A. MURRAY, III · D. L. WILLIAMS

ORGANIC SYNTHESES WITH ISOTOPES

PART I

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Organic synthese

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ORGANIC SYNTHESES WITH ISOTOPES, PART I

Page 73 In Note 2, line 5, read "converted, ^{7}".

Page 83 In C, line 2, read "C^{14}-bromocyclopentane".

Page 119 In C, line 5, read "bromoacetate-1-C^{14},".

Page 159 In line 1, delete "ethyl"

Page 196 In (a), line 1, insert "*Ethyl*".

Page 200 In alternate title read "...-3,4-$C^{13}_{1/2}$ Acid".

Page 210 In C, paragraph 3, line 1, read "L-5-ureido-$C^{14}_{1/1}$-3-$N^{15}_{1/1}$-norvaline".

Page 220 In C, line 2, read "...-4,5-C^{14}_{2},".

Page 296 In C, paragraph 3, line 1, read "L-Histidine...".

Page 311 In (b), line 1, read "...-6-*pteridylmethyl*-2-C^{14})*amino*..."

Page 357 In (b), line 1, read "5-(*carboxymethylene*) *hydantoin*-2-C^{14},".

Page 358 In (c), line 1, read "5-(carboxymethylene) hydantoin-2-C^{14},".

Page 359 In C, paragraph 2, lines 3 and 4, read "5-(carboxy-C^{14}-methylene) hydantoin".

Page 362 In C, lines 4 and 5, read as above.

Page 427 In (b), line 1, and in (c), line 2, read "*bicyclo*[2.2.1] *hept*-5-*en*-2-*yl*-2,3-C^{14}_{2},".

Page 431 In (a), line 1, read, "*2-Ethyl-1-hexanol-1*-C^{14},"

Page 459 In line 11, read "*o*-benzenediacetate-α,α'-C^{14}_{2},".

Page 459 In C, line 2, read "*o*-benzenediacetic-α-C^{14}_{1} acid".

Page 459 In C, line 3, read "benzenediacetate-α-C^{14}_{1}

Page 528 In Note 2, lines 3 and 4, read "2,3-di-4(or 5)-imidazolylpropionitrile-1-C^{14} hydrogen oxalate".

Page 552 In A, lines 13 and 14, read ", (7-acetamido-6,7-dihydro-1,2,3-trimethoxy-9-methoxy-C^{14}-benzo[*a*]heptalen-10(5*H*)one)".

Page 570 In C, line 18, read "triphenylmethylmagnesium".

Page 617 In lines 9 and 10, read "2,2,2-triphenylethanol-1-C^{14}".

Page 661 In Note 7, lines 3 and 4, read "2-chlorocyclohexanone".

Page 661 In Note 10, line 1, read "2,4-dinitrophenylosazone".

Page 744 In Note 10, line 1, read "α,α'-Dibromo-4-*t*-butylbibenzyl-α,α'-$C^{14}_{1/2}$".

Page 744 In Note 14, line 1, read "α,α'-Dibromo-3-methylbibenzyl-α,α'-$C^{14}_{1/2}$".

Page 777 In Procedure, lines 2 and 3 read "5-amino-1*H*-*v*-triazolo [*d*] pyrimidin-7-ol-3a C^{14},".

(see overleaf)

Page 780 In (a), line 2, read "*N*-*o*-toluidinemagnesium".

Page 816 In (b), line 19, read "hexahydrochloride".

Page 816 In C, line 2, read "hexahydro-".

Page 919 In line 1, read "2-methyl-1-propanol-3-C^{14},".

Page 938 In C, line 6, read "3-benzyloxy-1,2-propanediol-1-C^{14},".

Page 964 In C, line 2, read "3-chloro-1-propanol".

Page 969 In C, line 13, read "C_1^{14}-choline chloride".

Page 1061 In (a), lines 2 and 3, read "(methyl 17-carbomethoxy-5-oxo-3,5-seco-4-noretiocholan-3-oate),".

Page 1100 In (a), line 1, read "3-*(1,5-Dimethyl...*".

Page 1103 In (d), lines 2 and 3, read "*C^{14}-5-Oxo-5,7-seco-6-norcholestan 7-oic Acid)*".

Page 1104 In (e), line 1, read "*Methyl C^{14}-1-(1,5-Dimethyl...*".

Page 1104 In (e), line 2, read "*(Methyl C^{14}-5,7-Seco-6-norcholestan-7-oat*

Page 1104 In (f), line 2, read "*C^{14}-5,7-Seco-6-norcholestan-7-oic Acid)*".

Page 1104 In (g), line 9, read "C^{14}-1-(1,5-dimethyl...".

ORGANIC SYNTHESES WITH ISOTOPES

by Arthur Murray, III
and D. Lloyd Williams

Part I:
Compounds of Isotopic Carbon

Part II:
Organic Compounds Labeled with Isotopes of the Halogens, Hydrogen, Nitrogen, Oxygen, Phosphorus, and Sulfur

ORGANIC SYNTHESES WITH ISOTOPES

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PREFACE

This book is perhaps just as much a by-product of the atomic energy program as is a vast majority of isotopes, both stable and radioactive, which in the past several years have become available to research workers in the fields of chemistry, biology, medicine, agriculture and engineering. Scientists certainly have not been slow to appreciate the applicability of tracer methodology to all phases of organic chemistry and the life sciences.

Several thousand labeled organic compounds have been prepared, many of these by classical methods. Not infrequently, new methods of synthesis have been devised in order to gain an increased yield and to provide the most efficient utilization of the isotope.

Descriptions of the synthesis procedures which have been used are scattered throughout the literature; consequently workers must have access to a well-stocked reference library. In recent years the number of reports of new labeling procedures has diminished markedly because a majority of the low molecular weight compounds and many simple intermediates, which required special preparative techniques, have already been described. The present trend in publishing labeling procedures is to give references to earlier work as much as possible or to describe briefly, or merely outline, the additional steps required to obtain the desired product. For the above reasons, there seems to be a real need for a book which comprehensively collects and describes the scattered information pertaining to isotopic organic syntheses.

This compilation is an attempt to gather together all this material and present it as an encyclopedic source of methods of isotopic synthesis. The methods given are sufficiently detailed that they may be used directly in the laboratory and should prove of value in planning new isotopic preparations. An attempt has been made to treat all labeling syntheses mentioned in the literature through 1955.

We have described the preparation of only those compounds synthesized by chemical means. Biological procedures are not included, although data pertaining to exchange reactions are given, and many references are made to other work with isotopes which could be of use in the synthesis of labeled compounds. When several methods of labeling a particular compound were found in the literature, an attempt at evaluation was made, and the most feasible one was presented with inclusion of references to the others. This book should not be confined in its use to the preparation of labeled compounds alone, but it should be of considerable general

interest to the organic chemist because the procedures were usually chosen for or developed to give high yields.

The compilation of this material has emphasized to the authors the need for a uniform system of nomenclature for labeled compounds. It is hoped that the system used here, actually an extension of the nomenclature for nonisotopic compounds used by *Chemical Abstracts,* will contribute to the satisfaction of this need.

The authors wish to acknowledge their indebtedness to A. Weissberger and A. R. Ronzio, with whom the inception of this book originated. We are especially grateful to F. Newton Hayes and Donald G. Ott for a large part of the development of the complete isotopic organic nomenclature, which is used throughout this book. They also critically read the entire manuscript and made many valuable contributions and suggestions. We wish to thank Mrs. J. E. Furchner, Mrs. A. F. Malmberg and Miss Juanita Pino, who typed the entire manuscript in its many phases, and Mrs. W. H. Sullivan and Mrs. E. P. Nordberg, who provided clerical support. Mrs. Malmberg also aided materially in editing and proofreading the manuscript. We are grateful to Miss Gretchen Riese, who helped with the translation of articles in foreign journals, and to Mrs. J. O. Winks, who prepared the technical illustrations. We are greatly indebted also to Ralph Carlisle Smith and Leslie M. Redman of the Los Alamos Scientific Laboratory's Documentary Division for their help on a variety of technical and nontechnical problems.

We wish to express our appreciation to Wright H. Langham and Thomas L. Shipman, whose kind consideration, cooperation and encouragement made the preparation of this book possible.

This work was done under the auspices of the Atomic Energy Commission.

Los Alamos, New Mexico
March 11, 1957

A. M.
D. L. W.

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INTRODUCTION

A. ARRANGEMENT

As a result of the extensive utilization of isotopically labeled organic compounds as tracers in biological and medical research, and in the solution of industrial and purely academic problems, the number and variety of such compounds are necessarily large. The classification of these compounds by isotope has resulted in a logical presentation of the material in two volumes. Part I is composed entirely of the compounds of isotopic carbon. Part II contains organic compounds labeled with isotopes of hydrogen, the halogens, nitrogen, sulfur, phosphorus and oxygen.

Individual units of the text are composed of either one-step or multiple-step syntheses, and the title of each unit is the name of the end-product. The units are classified according to the labeling isotope. As indicated by the tables of contents, the units are grouped according to compound class designations, within which they are arranged according to parent structures in an increasing order of complexity. Since in any given synthesis procedure a number of labeled intermediates may be described but not represented in the synoptic tables, it cannot be overemphasized that the most efficient approach to the location(s) of a compound is through the combined index to Parts I and II. A compound which is the end-product in one synthesis may be the starting material in one or more other procedures; therefore, the preparation of an isotopic compound is indicated by a boldface page number in the index.

The section under each unit synthesis headed *Other Preparations* is rather broad in scope. It contains references to other methods of preparing the topic compound, references to similar compounds prepared essentially according to the procedures described, and, in some instances, to similar syntheses in which procedural details were lacking or inadequate.

Chemical compounds are indexed according to the Subject Index usage of *Chemical Abstracts* with the additional provision that isotopic designations appear as derivatives. The combined index to Parts I and II, with an outline of the indexing procedure, appears in Part II.

B. ISOTOPIC ORGANIC NOMENCLATURE

A collection of preparative procedures involving labeled organic compounds must, at the same time, be a collection of the names of these compounds. A book such as this which contains both a great number and a broad variety of isotopic names is an excellent proving ground for a systematic method of nomenclature. Inadequacies in one system become readily apparent under the test of repeated application; but another system which is clear and concise in its usage throughout the most difficult tests devisable for it will emerge as a welcome contribution.

Already-postulated rules for naming labeled organic compounds exist in the deuterium-tritium system of Patterson, Capell and Magill,[1] the carbon-isotope system of Collins, Crompton, Ronzio and Tolbert,[2] and in a few, less formal, published suggestions.[3,4]

The nomenclature developed for this book originated in these existing precepts, evolved during three years of study, and reached the present form when successfully applied to all names in this book.

The basic problem in isotopic nomenclature is the complexity of the molecular mixture which must be described in all stiuations involving dilute isotope and even in some cases the "pure" carrier-free nuclide. The convention for choosing the structure or combination of structures which must be named to characterize the labeled compound should constitute the first rule of the naming system.

Formulation of isotopic names is logically founded on acceptable nonisotopic nomenclature. The inherent difficulty here is the confusion of equivalent names resulting from so moderate a criterion as "acceptable." Another rule must either state the nonisotopic system of nomenclature to which one must adhere or admit chaos.

Further rules must define the mechanics for arriving at a proper isotopic name. Their invention must be governed by a requirement of general applicability and clarity along with a desire for simplicity. The fundamental decision was made for this nomenclature that simplicity would accompany the names and the mechanism for clarity would be written into the rules.

Rule I. THE ISOTOPIC NAME SHALL DESCRIBE THE *SINGLE STRUCTURE* OR *MIXTURE OF STRUCTURES*, EACH OF WHICH IS LABELED AT EVERY POSITION DICTATED BY THE PREPARATIVE METHOD FOR THE LABELED COMPOUND. THE ISOTOPIC NAME SHALL HAVE TWO PARTS: A NONISOTOPIC NAME FORMULATED IN ACCORDANCE WITH THE USAGE OF *CHEMICAL ABSTRACTS*, AND ONE OR MORE *ISOTOPIC DESIGNATIONS* WHICH PROVIDE ALL AVAILABLE INFORMATION ABOUT THE ISOTOPIC LOCATIONS IN THE LABELED COMPOUND.

This rule is based on the desire to keep the question of isotopic abundance completely separate from the assignment of names. This removes the temptation to focus attention on the most probable labeled structure and places proper emphasis on that structure or group of structures with highest isotopic multiplicity.

Exhaustive methylation of ammonia with methyl iodide whose carbon is 10% C^{14} will yield a mixture of five structurally distinguishable tetramethylammonium iodides. The composition of this mixture, assuming no isotope effects occur, can be calculated to be: 65.61% with no label, 29.16% with one label, 4.86% with two labels, 0.36% with three labels, and 0.01% completely labeled. These percentages depend on the original isotopic abundance in the methyl iodide. If the carbon of methyl iodide had been 90% C^{14}, the five quaternary salts would have been formed in just the opposite order of the same percentages. Application of Rule I indicates that this is a *single structure* case, regardless of the C^{14}-abundance of the methyl iodide, and that the molecule which must be described is tetramethylammonium iodide containing four C^{14} atoms.

2-Propanol labeled with C^{14} at one of the methyl groups dehydrates to give a mixture of propenes, one with C^{14} at the 1-position and the other with C^{14} at the 3-position. This is a *mixture of structures,* both of which may be described by a single isotopic name.

Artificial mixtures (mechanical, as distinguished from synthetic) may be described by a single isotopic name, when the rules permit.

This nomenclature has chosen to recognize two classes of the *mixture of structures.* The mode of distinction between these classes is characteristic of the nomenclature and not of the chemical structures involved.

A *class A mixture* is a *mixture of structures* in which every *isotopic designation* in its isotopic name completely describes one or more contributing structures. Almost every one of the mixture cases in this book is a *class A mixture.* A few examples are not in this category for two reasons: either there is a discontinuous numbering system in the nonisotopic name, or there are two or more different isotopes in the same structure.

A *class B mixture* is a *mixture of structures* which differs from a *class A mixture* in that it has a recurring label described by one or more *isotopic designations,* and takes the form of a *class A mixture* in the other *isotopic designations.* The *class B mixture* name gives the appearance of being derived from the mixture by factoring out a *single structure* and leaving a *class A mixture.*

This nomenclature does not provide a name for a mixture which is neither *class A* nor *class B.*

Rule I notes the dependence of this nomenclature on *Chemical Abstracts* as the most expedient source of nonisotopic names. Its subject index is not only a ready reference text for nomenclature but is also a repository of information on labeled compounds.

Rule II. THE COMPONENTS OF THE *ISOTOPIC DESIGNATION* SHALL BE SYMBOL, SUPERSCRIPT, SUBSCRIPT AND LOCANT. THE SYMBOL WITH ITS SUPERSCRIPT AND SUBSCRIPT IS SET OFF FROM OTHER PARTS OF THE ISOTOPIC NAME, EXCLUDING PUNCTUATION, BY MEANS OF HYPHENS. THE SYMBOL SHALL BE THE STANDARD CHEMICAL DESIGNATION OF THE ELEMENT. THE SUPERSCRIPT SHALL IMMEDIATELY FOLLOW THE SYMBOL AND SHALL BE THE MASS NUMBER OF THE ISOTOPE. WHEN *ISOTOPIC DESIGNATIONS* MUST APPEAR TOGETHER IN AN ISOTOPIC NAME, PRECEDENCE SHALL BE ESTABLISHED PRIMARILY BY ALPHABETICAL ORDER OF SYMBOL AND SECONDARILY BY NUMERICAL ORDER OF SUPERSCRIPT.

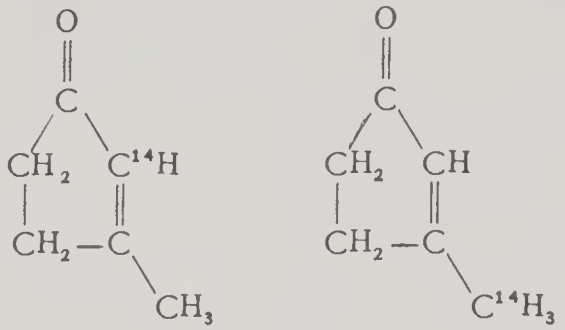
Rule III. THE SUBSCRIPT SHALL IMMEDIATELY FOLLOW THE SYMBOL AND SHALL BE AN INTEGER IN EVERY *ISOTOPIC DESIGNATION OF A SINGLE STRUCTURE* AND A FRACTION IN EVERY *ISOTOPIC DESIGNATION* OF A *CLASS A MIXTURE*. A *CLASS B MIXTURE* SHALL HAVE *ISOTOPIC DESIGNATIONS* WITH SUBSCRIPTS OF BOTH THE INTEGRAL AND FRACTIONAL VARIETY. THE INTEGRAL SUBSCRIPT SHALL STATE THE NUMBER OF LABELED ATOMS DESCRIBED BY ITS *ISOTOPIC DESIGNATION;* THE NUMERATOR OF THE FRACTIONAL SUBSCRIPT SHALL STATE THE NUMBER OF LABELED ATOMS IN EACH STRUCTURE DESCRIBED BY ITS *ISOTOPIC DESIGNATION;* AND THE DENOMINATOR OF THE FRACTIONAL SUBSCRIPT SHALL STATE THE NUMBER OF STRUCTURES DESCRIBED BY ITS *ISOTOPIC DESIGNATION.* THE INTEGRAL SUBSCRIPT "1" SHALL BE OMITTED EXCEPT IN CASES WHERE THE *ISOTOPIC DESIGNATION* DESCRIBES ONE OF TWO OR MORE EQUIVALENT LOCATIONS IN THE LABELED COMPOUND.

Examples of integral subscript usage in *single structure* cases are propane-1,2-C_2^{14} for $C^{14}H_3C^{14}H_2CH_3$ and semicarbazide-C^{13}-2-H^2-1,4-N_2^{15} for $N^{15}H_2NH^2C^{13}ON^{15}H_2$. This latter example is also an illustration of the proper order of *isotopic designations*. The discussion section of Rule V contains a name, cyclohexanecarboxylic-C^{14}-2-C^{13} acid, which is a required violation of the usual order of precedence.

It follows from Rule III that the sum of the implied and stated integral subscripts in the isotopic name of a *single structure* will be equal to the number of labeled sites in that structure.

Fractional subscript usage in *class A mixtures* is shown in the following three examples. Chlorination of naphthalene-1-C^{14} yields a mixture

of four compounds which is named 1-chloronaphthalene-1,4,5,8-$C^{14}_{1/4}$. 2,5-Hexanedione-1-C^{14} is cyclized to give the mixture:

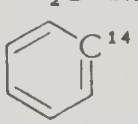


The name required is 3-methyl-$C^{14}_{1/1}$-2-cyclopenten-1-one-2-$C^{14}_{1/1}$. 2-Propanol-1-H^3_3 dehydrates to give propene-1-H^3_2 and propene-3-H^3_3. This *class A mixture* is named propene-1-$H^3_{2/1}$-3-$H^3_{3/1}$.

Nitration of benzene-C^{14}_1 with nitric-N^{15} acid, followed by reduction of the product to give aniline, yields a *class B mixture* of four compounds which is named aniline-$C^{14}_{1/4}$-N^{15}. Another example of a *class B mixture* is the combination of 1-ethyl-2-C^{14}-benzene-1-C^{14} and 1-ethyl-2-C^{14}-benzene-2-C^{14}. This is named 1-ethyl-2-C^{14}-benzene-1,2-$C^{14}_{1/2}$. The mixture, 1-ethyl-2-C^{14}-benzene-1-C^{14} and 1-ethyl-1-C^{14}-benzene-2-C^{14}, cannot be given an isotopic name with this nomenclature because of the partially repetitive numbering system of ethylbenzene. If the two structures of the last mixture were dehydrogenated to styrenes, a *class A mixture* would result, thanks to the nonrepetitive numbering system of styrene. The name, the isotopic locant system of which will be evident after Rule IV, is styrene-(α,2), (β,1)-$C^{14}_{2/2}$.

Another consequence of Rule III is that the sum of the denominators of the fractional subscripts in the isotopic name of a *class A mixture* or a *class B mixture* will be equal to the number of structures in that mixture. It also follows that the sum of the numerator-denominator products of the fractional subscripts in the isotopic name of a *class A mixture* will be equal to the number of labeled atoms in all the contributing structures. These corollaries are useful in checking the correctness of a name.

The correct use of the subscript "1" is demonstrated in the following examples:

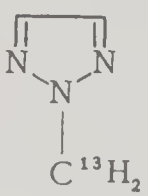
$N^{15}H_2CONH_2$	urea-N^{15}_1
	benzene-C^{14}_1
$CHH^3{=}CH_2$	ethylene-H^3_1
$C^{13}H_2{=}CH_2$	ethylene-C^{13}_1
$C^{14}H_2{=}CHH^2$	ethylene-1-C^{14}-2-H^2_1

Rule IV. THE LOCANTS SHALL PRECEDE THE SYMBOL AND SHALL INDICATE THE LOCATIONS OF THE LABELED ATOMS. LOCANTS FOR *ISOTOPIC DESIGNATIONS* SHALL BE IDENTICAL IN KIND AND USAGE TO THOSE IN NONISOTOPIC NAMES. WHENEVER NO LOSS OF INFORMATION RESULTS FROM OMISSION OF LOCANTS, THE RESULTING SIMPLIFIED NAME SHALL BE USED. WHENEVER BOTH THE NUMERATOR AND THE DENOMINATOR OF THE SUBSCRIPT IN AN *ISOTOPIC DESIGNATION* OF A *MIXTURE OF STRUCTURES* ARE GREATER THAN 1, THE LOCANT AGGREGATE SHALL BE CONSTRUCTED OF TWO OR MORE GROUPS OF LOCANTS WITHIN PARENTHESES, THE NUMBER IN EACH GROUP BEING EQUAL TO THE NUMERATOR.

There are three classes of locants: stated, implied, and virtual. Stated locants are those expressed as numbers, Greek letters or capital-letter element designations. Implied locants are those omitted according to Rule IV. Virtual locants are missing because of lack of a locant designation in the nonisotopic name. The name 1-methyl-C^{14}-piperidine-4-C^{14}-N^{15} contains all the locant types in the order virtual, stated and implied.

There are two varieties of locant usage, one involving (a) the position at which the isotope is situated, and the other (b) the position to which the isotope is attached. This dual nature of the locant is illustrated in the following examples, after which are employed the preceding code letters for locant varieties:

$CH_3C^{14}H_2CH_3$	propane-2-C^{14}	(a)
$CH_3CH_2^2CH_3$	propane-2-H_2^2	(b)
$CH_3NHCON^{15}H_2$	1-methylurea-3-N^{15}	(a)

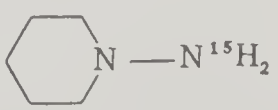


2-methyl-2*H*-1,2,3-triazole-2-C^{13} (b)

or

2-methyl-C^{13}-2*H*-1,2,3-triazole (a)

All of these examples are strictly correct. It is traditional, however, always to employ locant type (a) for carbon isotopes. With isotopes other than carbon, ambiguity is rarely encountered. With a case such as



locant usage of type (b) gives the incorrect name: 1-aminopiperidine-1-N^{15}. 1-Amino-N^{15}-piperidine is required and uses locant type (a).

The second sentence of Rule IV is a re-emphasis of that part of Rule I pertaining to nonisotopic nomenclature. *Chemical Abstracts* uses locants in most of its major names, as evidenced by derivatives which are listed in subheadings as position-numbered substituent groups.

Other major names have no locant numbers, as inferred from the statement under their names: "for derivatives, see ....." According to Rule IV, if there is no 2-bromoisobutyric acid, then there is no "isobutyric-2-C^{14} acid," etc. Following the usage of *Chemical Abstracts*, this *isotopic name* should be 2-methylpropionic-2-C^{14} acid. "Stearic-2-C^{14} acid" should be octadecanoic-2-C^{14} acid, and "adipic-1,6-C_2^{14} acid" should be hexanedioic-1,6-C_2^{14} acid.

The anhydride of 2-chloropropionic acid is known as bis(2-chloropropionic) anhydride. Similarly, this nomenclature names the anhydride of propionic-2-C^{14} acid, bis(propionic-2-C^{14}) anhydride. Completely analogous cases are *N,N*-bis(ethyl-1-C^{14})aniline and tris(propyl-2-H_2^3) citrate.

Names like chlorobenzene, phenylhydrazine and phenylalanine do not have locants, as evidenced by the necessity to use *two* locants to describe their singly substituted derivatives. Isotopic names taking this into consideration are: 1-chlorobenzene-4-H^2, 1-phenylhydrazine-1-N^{15}, and 3-phenylalanine-1,2-C_2^{14}.

The third sentence of Rule IV allows the following simplified names: propane-C_3^{14} instead of propane-1,2,3-C_3^{14}; benzene-C_5^{14} instead of benzene-1,2,3,4,5-C_5^{14}; cyclopentane-1,2,4-H_6^3 instead of cyclopentane-1,1,2,2,4,4-H_6^3; propene-1-$H_{2/1}^3$-3-$H_{3/1}^3$ instead of propene-1,1-$H_{2/1}^3$-3,3,3-$H_{3/1}^3$; and aniline-$C_{1/4}^{14}$-N^{15} instead of aniline-1,2,3,4-$C_{1/4}^{14}$-N^{15}. The name "acetone-H_6^2" is not allowed because it is a contraction of the incorrect name "acetone-1,3-H_6^2." Acetone has no locants, and the correct name is 2-propanone-H_6^2.

The last sentence of Rule IV establishes a basis for the form used for a multiply-labeled *mixture of structures*. The mixture

$CH_2{=}C^{14}HC^{14}H_2CH_2CH_2CH_2OH$ and $CH_2{=}CHCH_2C^{14}H_2C^{14}H_2CH_2OH$

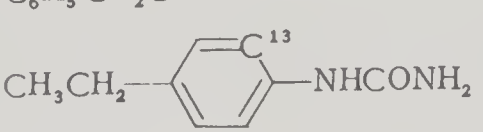
is named 5-hexen-1-ol-(2,3),(4,5)-$C_{2/2}^{14}$. The chlorination product of benzene-1,2,3-C_3^{14} is a mixture of four structures and is named 1-chlorobenzene-(1,2,3),(1,2,6),(2,3,4),(3,4,5)-$C_{3/4}^{14}$.

Before this discussion of components of the *isotopic designation* is ended, a frequently used naming method which circumvents these rules must be mentioned. This is a purely descriptive means of locating the isotope which, for example, gives the singly O^{18}-labeled structures of ethyl acetate the names: ethyl acetate carbonyl-O^{18} and ethyl acetate alcohol-O^{18}. This book prefers to avoid all such descriptive *isotopic designations* and names these ester cases 3-oxa-2-pentanone-2-O^{18} and 3-oxa-2-pentanone-3-O^{18}.

Rule V. WHENEVER THE POINT OF LABEL CAN BE LOCATED IN THE NONISOTOPIC NAME, THE *ISOTOPIC DESIGNATION* SHALL FOLLOW THAT PART OF THE NONISOTOPIC NAME WHICH IT MODIFIES. THE PREFERRED POSITION FOR THE *ISOTOPIC DESIGNATION* SHALL BE AT THE END OF A WORD. WHENEVER LOCANT AMBIGUITY MAKES THIS IMPOSSIBLE, THE *ISOTOPIC DESIGNATION* SHALL APPEAR AT THE END OF THE RADICAL IN WHICH THE LABEL RESIDES. WHENEVER THE LOCANT OF AN *ISOTOPIC DESIGNATION* DOES NOT DEFINE WHICH OF TWO OR MORE PRECEDING SECTIONS OF THE ISOTOPIC NAME IS MODIFIED BY THAT *ISOTOPIC DESIGNATION*, THE *ISOTOPIC DESIGNATION* SHALL APPLY TO THAT SECTION IMMEDIATELY PRECEDING IT. WHENEVER THE LABEL IS IN A SUBSTITUENT GROUP WHICH HAS NO POSITION NUMBER AND WHICH APPEARS IN THE NAME OR RADICAL AS A FUNCTIONAL GROUP SUFFIX, PLACEMENT OF THE *ISOTOPIC DESIGNATION*, WITH NO LOCANT, IMMEDIATELY AFTER THE NAME OR RADICAL SHALL BE TAKEN TO MEAN LOCATION OF THE LABEL IN THAT SUBSTITUENT GROUP.

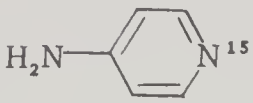
Rule V clearly separates isotopic from nonisotopic usage for the substituent or modifying group. Complexity in the rules is required to bring about simplicity in the isotopic name which carries a subsequent *isotopic designation*.

When the point of label can be located in the nonisotopic name, there are four discernible variations of the placement of the *isotopic designation*: (a) end of word and no locant ambiguity; (b) end of radical and no locant ambiguity; (c) end of word and more than one possible preceding location; and (d) end of radical and more than one possible preceding location. One example of each type follows, along with the code letters:

$C^{14}H_3NHNH_2$	methylhydrazine-C^{14}	(a)
$C^{13}H_3NHCONH_2$	methyl-C^{13}-urea	(b)
$C_6H_5CH_2C^{14}OOH$	phenylacetic-1-C^{14} acid	(c)
	(*p*-ethylphenyl-2-C^{13}) urea	(d)

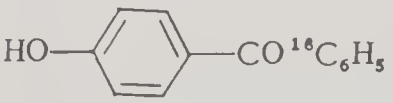
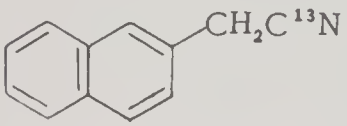
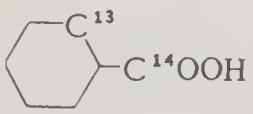
The third sentence of Rule V removes the ambiguity from names of types (c) and (d). This might also have been done by the use of parentheses: phenyl(acetic-1-C^{14}) acid and [*p*-ethyl(phenyl-2-C^{13})]urea. However, proper use of Rule V makes these parentheses unnecessary.

Improper use of the rule could give the structure



the incorrect name, 4-aminopyridine-N^{15}, with the thought that the N^{15} applies to the immediately preceding pyridine nitrogen and not to the amine nitrogen. The correct name, using the available locant, is 4-aminopyridine-1-N^{15}.

Rule V provides for the many cases in which the label has no assignable locant. Examples of the useful application of this rule follow:

$C_6H_5C^{14}OOH$	benzoic-C^{14} acid
$CH_3CH_2OH^3$	ethanol-H^3
	4-hydroxybenzophenone-O^{16}
	2-naphthaleneacetonitrile-C^{13}
	cyclohexanecarboxylic-C^{14}-2-C^{13} acid

Rule VI. WHENEVER THE POSITION OF LABEL IS UNKNOWN OR WHENEVER NO LOCANT USAGE IS POSSIBLE, THE *ISOTOPIC DESIGNATION* SHALL PRECEDE THAT PART OF THE NONISOTOPIC NAME WHICH IT MODIFIES. WHENEVER THIS INDEFINITE *ISOTOPIC DESIGNATION* APPEARS WITHIN A WORD, CLARIFYING PARENTHESES MUST BE USED TO CONNECT IT WITH THE INVOLVED SUBSEQUENT PORTION OF THE NONISOTOPIC NAME.

Rule VI allows for indefinite naming with an *isotopic designation* from which locants necessary for complete clarity are missing. This condition may be due to a trivial name without locants or to a case in which the location of labeling is in doubt. If subscript information is known, it is given according to Rule III. Representative examples are H^3_4-hexestrol and C^{14}_1-erythromycin.

Rule VI provides that there shall be no confusion with Rule V when the indefinite *isotopic designation* occurs in the middle of a word. 1-Methyl-C^{14}-naphthalene is labeled in the methyl group, and 1-methyl(C^{14}-naphthalene) is labeled an unknown number of times at unknown positions in the naphthalene ring.

C. PROPERITIES AND PROCUREMENT OF ISOTOPES

A number of factors must be considered in the selection of a suitable isotope for labeling a particular organic compound. The most obvious are those imposed by the composition of the compound, its intended use and the chemical difficulties of introducing the isotope. Other factors which must be considered are: availability and half-life of the isotope, specific activity desired, the sensitivity of available methods of detection or assay, stability of the labeled molecule and cost. Physical data and other information that should be useful in selection of an isotope for a particular labeling problem are given in Table I, 1.

Comar[5] has presented specific information on a number of isotopes which are useful experimentally in biology and agriculture. These data include availability, physical properties, methods of assay, chemistry involved in isotopic detection, and typical experimental procedures for the use of isotopes. A comprehensive compilation of useful data, including the availability of radioactive and nonradioactive isotopes and of labeled compounds, both inorganic and organic, has been presented in the Isotope Index.[6] Information on prices, availability, procurement procedures, and properties of radioactive isotopes is given in the Oak Ridge National Laboratory's Catalog and Price List.[7]

D. SOME SPECIALIZED TECHNIQUES IN ISOTOPIC SYNTHESES

1. Semimicro Techniques and Vacuum Systems

A considerable number of standard reactions and practices of organic chemistry have been used in synthesizing labeled compounds. However, most of these have been modified in order to increase the yields and decrease the scale of essential operations.

In general, the synthesis of compounds labeled with stable isotopes such as C^{13}, H^{2}, N^{15} and O^{18} can be accomplished in the usual type of laboratory glassware. However, the use of semimicro techniques and equipment is often advantageous because of ease of handling, conservation of the compounds involved and the time consumed in making the synthesis. Micro and semimicro equipment and methods are essential to the synthesis of radioactively labeled compounds in which high specific activities are desired. Such methods and their application to isotopic synthesis are discussed in detail by others.[8,9]

The synthesis, manipulation and storage of small amounts of radioactive materials, especially those of low molecular weight and high volatility, can usually be accomplished most efficiently and safely in vacuum-type apparatus. The nature of the reactions and materials frequently requires inert atmospheres, and it is not unusual to use a vacuum manifold to carry out several steps of a multiple-step synthesis

involving numerous transfers. A number of figures demonstrating versatile and unusual designs of vacuum systems and semimicro apparatus used in isotopic syntheses are dispersed throughout the text.

Information on the design and manipulation of high vacuum apparatus[8,9] has been presented in numerous textbooks.

2. Methods of Isotopic Measurement

Labeled organic compounds are always synthesized for the specific purpose of using the measurement of the isotope as a means of quantitation. Although the organic chemist may not be the ultimate user of a labeled compound, he may find it essential to make isotopic measurements in studies of reaction mechanisms, in following the course of a labeling synthesis, or in providing an isotopic assay of the final product.

a. Stable Isotopes

Some methods now available for determination of stable isotopes require only apparatus and instruments which are generally available in many chemical laboratories, and the procedures are seldom more complicated than ordinary analytical techniques. All isotopes of an element can be measured on the basis of mass ratios, and the mass spectrometer provides the most generally applicable method. Conversion of the material being analyzed to the analytical sample depends on its nature and on the elemental species of the isotope. Often the conversion methods applicable to radioactive isotopes are adaptable also to stable isotopes and *vice versa.*

Deuterium.—Assay of the stable hydrogen isotope generally proceeds from the initial conversion of the sample to water by Pregl technique or by sealed-tube combustion.[11,20–22] The water may then be analyzed for deuterium content by infrared spectroscopy,[20,21,23] optical spectroscopy,[24] or density determination by the falling drop method.[11,22,25,26] The water may be converted to hydrogen[11,27,28] or to a hydrocarbon[11,29,30] and mass spectrometric methods applied.

Carbon-13.—Analysis for C^{13} invariably involves conversion of the sample to carbon dioxide, which then can be subjected to mass spectrometric methods[11,31,32] or infrared gas analysis,[23,33] or converted to acetylene for analysis by a potentially useful method of flame spectrophotometry.[34]

Nitrogen-15.—Nitrogen-15 may be analyzed in the mass spectrometer in the form of nitrogen gas which is obtained by the Dumas method,[11,35] by ampoule combustion,[31,32,36] or by hypobromite oxidation of ammonia from the Kjeldahl method.[11,37,38] Infrared gas analysis of nitrous oxide[33] and ultraviolet photometry of nitrogen gas excited by electrodeless discharge[39] have been described.

TABLE I, 1

Isotopes of Elements Most Common to Organic Compounds

Isotope	Half-life or % nat. abundance	Modes of decay and energy of radiations (Mev)		Form supplied	Specific act. or conc.
		beta[a] (%)	gamma		
H^2	0.0156%	–	–	gas or water	99.6%
H^3	12.26 yr.	β^- 0.0176	none	gas, carrier-free	2.95 c./cc. at STP
C^{11}	20.4 min.	β^+ 0.968	none	–	–
C^{12}	98.89%	–	–	C	99.9–99.96%
C^{13}	1.11%	–	–	C, $BaCO_3$	69%, 60%
C^{14}	5600 yr.	β^- 0.1585	none	$BaCO_3$	160–1200 mc./g. C
N^{15}	0.365%	–	–	NH_4Cl	60%
				NH_4NO_3	7–60%
				NH_4OH	60%
O^{18}	0.206%	–	–	water	10%
F^{18}	112 min.	β^+ 0.65	none	fluoride soln.	–
P^{32}	14.3 d.	β^- 1.712	none	H_3PO_4 in HCl soln.	~40 c./g. P
				H_3PO_4 in HCl soln.	carrier-free
S^{35}	87.1 d.	β^- 0.165	none	H_2SO_4 in HCl soln.	carrier-free
				BaS in $Ba(OH)_2$ soln.	>10,000 mc./g. S
				S in benzene soln.	>1000 mc./g. S

Isotope	Half-life or % nat. abundance	Modes of decay and energy of radiations (Mev)		Form supplied	Specific act. or conc.
		beta[a] (%)	gamma		
Cl^{35}	75.53%	–	–	NaCl, AgCl	89.7–95.7%
Cl^{36}	3.08×10^5 yr.	β^- 0.714 (98%)	–	HCl	>0.1 mc./g. Cl
Cl^{37}	24.4%	–	–	NaCl, AgCl	60.5–66.3%
Cl^{38}	1 sec.	–	0.66[b]	–	–
Cl^{38}	37.3 min.	β^- 4.81 (53%)	–	–	–
		β^- 1.11 (31%)	1.60, 2.15	–	–
		β^- 2.77 (16%)	2.15	–	–
Br^{80}	4.58 hr.	–	0.049[b]	–	–
Br^{80}	18.5 min.	β^- 1.99 (85%)	–	–	–
		β^- 1.38 (15%)	0.620	–	–
Br^{82}	25.7 hr	β^- 0.465	0.535, 0.750 1.02	KBr in HCl soln.	>1000 mc./g. Br
I^{128}	25.0 min.	β^- 2.12 (76%)	–	–	–
		β^- 1.67 (16%)	0.46	–	–
I^{129}	1.72×10^7 yr.	β^- 0.15	0.038	NaI in basic Na_2SO_3	0.2 μc./mg. I
I^{131}	8.05 d.	β^- 0.606 (87%)	0.364 (81%)	NaI in basic Na_2SO_3	carrier-free

[a] Expressed as maximum energy
[b] Isomeric transition

Oxygen-18.—The mass spectrometric method has almost completely supplanted that depending on the determination of density differences of water.[40] Water is assayed by equilibration with carbon dioxide, which is then used for determination of the isotopic ratio.[41,42] The method of Schütze-Untersaucher[43] for the determination of oxygen in organic compounds by conversion to carbon dioxide has been adapted for isotopic purposes.[44,45] Ampoule combustion techniques may also be employed.[31,32] Equilibration of a variety of organic substances with carbon dioxide by use of a sulfuric acid catalyst has been found possible.[46] It seems likely that infrared spectral methods may soon be applied to routine determinations.[40]

b. Radioactive Isotopes

(1) Assay Methods.—The quantitative determination of radioactive isotopes is based on well known methods of detecting the energetic particles they emit during radioactive decay. Ionization chambers, Geiger counters, proportional counters and various modifications of scintillation detectors (liquid, plastic and crystal) are the usual detectors employed. No single device is best for measurement of radiations of all types, but each instrument has its particular sphere of usefulness. Table I, 2 summarizes the types of detectors and their applications to the assay of labeled compounds.

The choice of an assay method for a radioactive isotope depends on a number of factors, the most important of which are the type and energy of the emitted radiation. Isotopes that emit weak beta particles (e.g., H^3, C^{14}, S^{35}) are best measured by methods that maximize geometrical efficiency and minimize absorption of the radiations in the sample and in the walls of the detector. Gas-phase Geiger and proportional counters, ionization chambers (into which the sample is introduced as a gas), and more recently, various modifications of the liquid scintillation detector appear to be the methods of choice for samples having relatively low specific activities. If the specific activity of the sample is adequate, weak beta-emitting isotopes can be assayed in solid and liquid phase with internal proportional and Geiger counters and with conventional thin-window Geiger tubes.

Isotopes that emit energetic beta particles (e.g., P^{32}, F^{18}, Cl^{36}, I^{131}) may be measured with any of the detectors mentioned previously. The choice of method may be made on the basis of availability and convenience. Geiger and proportional counters are most frequently used.

Gamma-emitting isotopes (e.g., I^{131}, Br^{82}, Cl^{38}) can be measured efficiently with the sodium iodide (thallium-activated) crystal scintillation counter. Good efficiency is obtained as a result of the high stopping power of the sodium iodide, and excellent energy resolution results from the significant fraction of gammas which enter into the photoelectric process. Equipped with a double gate discrimination circuit, this de-

tector is a gamma-ray spectrometer which provides a method of isotopic identification and multiple-isotopic analysis. Because of the high penetrating power of gamma rays, gamma-emitting isotopes usually can be assayed in liquid or solid state without isolation or prior chemical processing of the labeled material.

Liquid scintillation counting.—Liquid scintillators are organic solutions (usually three-component systems consisting of a solvent and primary and secondary solutes) which emit light by their interaction with ionizing radiation. The emitted light is absorbed by the photocathode of a photomultiplier tube, and the photo-electrons are multiplied, amplified, sorted and then counted by a conventional scaling circuit.

The most efficient solvents are alkylbenzenes (e.g., toluene).[96] A double-solvent system, consisting of *p*-dioxane and naphthalene, is very useful for internal counting of tritium water and various aqueous solutions.[51] The best primary and secondary solutes are polyaryl compounds made up of aromatic rings linked in a linear configuration [e.g., *p*-terphenyl and 2,2′-*p*-phenylenebis(5-phenyloxazole) (POPOP)].[97,98] Dissolution of the sample in the scintillator solution affords high total counting efficiency through good geometry and low self-absorption in the sample.

The liquid scintillation counter affords a rapid, efficient, and versatile means for assay of organic and inorganic compounds, as well as biological materials, labeled with any radioactive isotope. High total efficiency and ease of sample preparation frequently make this method especially attractive for the assay of materials labeled with H^3, C^{14} and S^{35}. In most cases, a system can be used in which the organic compound is soluble; when this is not possible or convenient, a suspension of the substance in the liquid[69] or a thixotropic-gel[71] scintillator gives very good results except with tritium-labeled materials. In the latter case self-absorption of the very weak beta rays in the particles of the suspension is too great. Compensation for variations such as quenching effects can be made by addition of an internal standard after the sample has been counted. The method is also capable of simultaneous analysis of two or more radioactive isotopes, provided there is sufficient difference in the energy spectra of their radiations. Recent comprehensive articles regarding the liquid scintillation counter are available.[99–101]

Radioactive standards and absolute measurements.—Determination of the absolute disintegration rate of a radioactive sample is not necessary in many experiments. Usually, the ratio of activities before and after a particular conversion or operation is the result sought. The relative counting efficiency may be determined in this case by use of a suitable standard sample. There are, however, occasions when an absolute determination of the isotopic concentration is necessary (e.g., in determining absolute specific activity in compliance with specifications, or for the calculation of radiation dose). Such absolute measurements may

be made by comparison of the counting rate of the sample (under identical counting conditions) with that of a standard whose absolute disintegration rate is known. Standards of known disintegration rates can be prepared from isotopic mixtures that have been carefully analyzed by the mass spectrographic method. The preparation of benzoic-C^{14} acid as a standard for liquid scintillation counting has been reported.[102] The National Bureau of Standards is actively engaged in a program of preparing isotope standards, a few of which (including I^{131}, P^{32}, H^3, and C^{14}) are already available.

(2) Preparation of Samples.—A major factor in the assay of many radioactively labeled organic compounds is their conversion to proper chemical form for counting. The nature of the compound and the energy of radiation emitted by the labeling isotope determine the necessity for conversion to a suitable analytical sample, and determine also the characteristics such a sample must have. These factors frequently determine the choice of detector to be used.

Compounds labeled with tritium are usually converted to water-H_2^3, which is then assayed or further converted to hydrogen-H_2^3 or a low molecular weight hydrocarbon. The compound also may be counted directly by use of the liquid scintillation counter. Compounds labeled with radioactive carbon are usually counted directly in the liquid scintillation system, converted to low molecular weight hydrocarbons for gas-phase assay, or converted to carbon-C^{14} dioxide, which is then assayed directly or converted to either barium carbonate-C^{14} or some other derivative for solid-phase measurements. Compounds labeled with S^{35} may be counted directly in various detectors or converted to a derivative (e.g., barium sulfate-S^{35} or benzidine sulfate-S^{35}) for solid-phase measurements. Materials labeled with isotopes that emit energetic beta particles or gamma rays are usually counted directly in either the solid or liquid phase. Methods of assaying radioactively labeled compounds, including intermediate and analytical samples (when necessary), and the form in which the samples are counted are summarized in Table I,2.

3. Chromatography

In order that valid conclusions may be drawn from the use of a radioactive tracer, it is necessary to establish conclusively that the isotope measured belongs exclusively to the compound itself and is not present as a trace contaminant.

The presence of small quantities of radioactive by-products or breakdown products in an organic compound cannot be determined by the classical criteria[103] of chemical purity nor by the usual chemical and physical tests. Such labeled impurities constitute a negligible fraction of the total material but may account for a significant fraction of the total radioactivity. The most satisfactory method of establishing the

TABLE I, 2

Assay of Radioactively Labeled Organic Compounds

Labeling isotope	Intermediate compound (when necessary)	Analytical sample (when necessary)	Sample form	Detector
H^3	–	–	direct plate	prop. flow or Geiger ctr.[47]
H^3	–	–	solute	liquid scint.[48,49]
H^3	–	H_2^3O	gas filling	Geiger ctr.[11,50]
H^3	–	H_2^3O	solute	liquid scint.[51]
H^3	–	$H_2^3 + CH_4^3$	gas filling	ion. chbr. with electrometer[11,52]
H^3	H_2^3O	H_2^3	gas filling[11]	ion. chbr. with electrometer[53–55]
H^3	H_2^3O	H_2^3	gas filling (CH_4 or argon)	Geiger ctr.;[56] prop. ctr.[11,57]
H^3	H_2^3O	CH_4^3 [58] or CH_3H^3 [59]	gas filling[11]	Geiger ctr.; prop. ctr.
H^3	H_2^3O	n-$C_3H_7CH_2H^3$	gas filling	Geiger ctr.[60]
C^{14}	–	–	external sample	ion. chbr. with electrometer;[61] geiger ctr.[62–64]
C^{14}	–	–	solute	liquid scint.[49,65–68]
C^{14}	–	–	suspension	liquid scint.;[69] gel scint.[70,71]
C^{14}	–	$C^{14}O_2$	gas filling[11]	ion. chbr. with electrometer[72–74]
C^{14}	–	$C^{14}O_2$	gas filling	prop. ctr.[11,75]
C^{14}	–	$C^{14}O_2$	gas filling (CH_4)	prop. ctr.[11,57]
C^{14}	–	$C^{14}O_2$	gas filling (CS_2)	Geiger ctr.[11,76]
C^{14}	–	$C^{14}O_2$	gas filling (argon and CH_4)	Geiger ctr.[77,78]
C^{14}	$C^{14}O_2$	C^{14}	internal plate	screen-wall Geiger ctr.[79]
C^{14}	$C^{14}O_2$	$C^{14}H_4$	gas filling	prop. ctr.[80]
C^{14}	$C^{14}O_2$	$C^{14}H_3OH$	solvent	liquid scint.[81]
C^{14}	$C^{14}O_2$	$C_2^{14}H_2$	gas filling	prop. ctr.[11,82]
C^{14}	$C^{14}O_2$	$C_6H_5C^{14}H_3$	solvent	liquid scint.[81]
C^{14}	$C^{14}O_2$	$BaC^{14}O_3$	plate[8]	flow ctr.; Geiger ctr.
C^{14}	$C^{14}O_2$	$BaC^{14}O_3$	suspension	liquid scint.[69]
C^{14}	$C^{14}O_2$	$R_3\overset{+}{N}C^{14}O_2^-$	solute	liquid scint.[83]
	$C^{14}O_2$, $C_2^{14}H_2$	hexane-C_6^{14} and octane-C_8^{14}	solvent	liquid scint.[84,85]
C^{14} and H^3	–	$C^{14}O_2$ and H_2^3O	see preceding methods	

(continued)

TABLE I, 2 (*continued*)

Labeling isotope	Intermediate compound (when necessary)	Analytical sample (when necessary)	Sample form	Detector
C^{14} and H^3	–	–	solute	liquid scint.
P^{32}	–	–	external sample	Geiger ctr.[86]
P^{32}	–	–	external sample	crystal scint.[87]
S^{35}	–	–	internal sample	prop. flow or Geiger ctr.[88]
S^{35}	$S^{35}O_4^{=}$	$BaS^{35}O_4$	plate[89]	prop. flow ctr.; Geiger ctr.
S^{35}	$S^{35}O_4^{=}$	$BaS^{35}O_4$	suspension	liquid scint.[69]
S^{35}	$S^{35}O_4^{=}$	benzidine sulfate-S^{35}	plate[90]	Geiger ctr.[91]
S^{35}	$S^{35}O_4^{=}$	benzidine sulfate-S^{35}	suspension	liquid scint.[69]
Cl^{36}	–	$Hg_2Cl_2^{36}$ or $AgCl^{36}$	plate[92]	prop. ctr.; Geiger ctr.
I^{131}	–	–	–	Geiger ctr.; crystal scint.[93]
Gamma emitters	–	–	external sample	crystal scint.[98,95]

radiopurity of a compound is its resolution into pure chemical species by some chromatographic method with subsequent determination of their radioactive content. In general, the most convenient technique is that of paper chromatography and radioautography;[104–107] if only one spot is observed on the X-ray film, a high degree of radiopurity is indicated. Obvious advantages of this method are the sensitivity with economy of material and the measure of autodecomposition given by comparison of original radioautographs with those prepared at a later date.[108–110]

The field of chromatography is well covered today by books,[10,111–123] review articles,[124–130] bibliographies[131] and symposia.[132,133] Annual and biannual review articles such as those in the January issue of *Analytical Chemistry,* serve to keep one abreast of current developments. The radioautographic process has been fully discussed by Boyd.[134]

E. ISOTOPE EFFECT

The term *isotope effect* is used to designate the isotopic fractionation which takes place in intermolecular and intramolecular chemical transformations due to the effect of mass difference upon reaction rates and/or equilibria. In general, the higher the atomic weight of the element, the less will be the fractional mass difference between the isotopes, and consequently the less will be the isotope effect. Isotopes of hydrogen represent the extreme case.

The effect of C^{13}, C^{14}, N^{15} and O^{18} substitution on organic reaction rates has been considered by Ropp,[135] and reviews of isotope effects in

chemical reactions have been published by Yankwich,[136] Wiberg[137] and Promislow.[138]

Although the isotope effect results in products with isotopic ratios different from those of the precursors, it is usually of minor consequence in synthetic work, especially when the yields are almost quantitative or when the isotopic atom is not involved directly in bond making or breaking. Greater importance of this effect may be expected in experiments involving diffusion-like processes, especially when lighter elements are involved and very accurate measurements are required. This effect is of considerable significance in evaluating degradations and reaction mechanisms.

The implications of the isotope effect in biological experiments are summarized by Aronoff[139] and Comar.[140]

F. AUTODECOMPOSITION OF COMPOUNDS LABELED WITH RADIOACTIVE ISOTOPES

The concentration of radioisotope desired in a labeled compound depends on the isotopic dilution which will be encountered in the over-all experimental system in which the compound is used and on the efficiency of the analytical method. For organic reaction mechanism studies or isotope dilution analyses, the concentration of isotope desired is generally several orders of magnitude less than that required with biological systems in which large dilution factors appear. Autodecomposition of radioactively labeled compounds occurs as a consequence of self-absorption of the emitted radiations, and of the change in elemental species attending isotopic decay. Although the second factor is usually insignificant, the first should be considered carefully when experiments involving high specific activities and long storage periods are contemplated.

The changes produced in a great many classes of pure organic compounds by the action of ionizing radiation have been reviewed extensively.[141-144] The self-radiolysis of a number of C^{14}-labeled compounds of high specific activity has been reported;[109, 110, 145-148] an extremely high rate of decomposition has been observed[149] in a preparation of succinic-H^3_4 acid with a specific activity of about 120 curies per mmole; and the decomposition of α-lipoic-S^{35}_{22} acid (0.3% per day) has been investigated.[150]

Calculations indicate that autodecomposition of C^{14}-labeled compounds having a specific activity of 1 mc./mmole should occur at the rate of about 0.3% per year.[145] Occasionally, however, the decomposition appears to proceed at an accelerated rate through a radical or ionic chain reaction[109,110,148](Table I,3). The products formed as a result of radiodecomposition may be many and varied, since the reactions produced may involve polymerization, oxidation, reduction and rearrangement.

TABLE I, 3

Autodecomposition of Some C^{14} Compounds

Compound	Decomposition			Radiation		
	Obs. (%)	Calc. (%)	Molecules per 100 e.v.	dose rep. $\times 10^{-6}$	sp. act. (mc./mmole)	approx. time (mos.)
(2-Hydroxyethyl) trimethyl-C_1^{14}-ammonium chloride	63	0.4	490	10.7	1.81	8.8
(2-Hydroxyethyl) trimethyl-$C-_1^{14}$ ammonium chloride	–	–	1780	2.5	0.08	46
(2-Hydroxyethyl) trimethyl-$C-_1^{14}$-ammonium chloride	–	–	1490	1.5	1.50	0.67
Cholesterol-4-C^{14}	40	–	–	7	2.51	17.4
Cholesterol-26-C^{14}	32	–	–	–	0.59	24
Calcium glycolate-2-C^{14}	22	0.9	77	17.5	0.76	46
Calcium glycolate-1-C^{14}	13	1.3	31	25.4	1.10	46
Methanol-C^{14}	19	5.1	12	593	8-10	21
Cholesterol-24-C^{14} (under vacuum)	7	–	–	–	0.07	15
Norvaline-3-C^{14} hydrochloride	5	1.7	9	40.3	2.72	24
(2-Acetoxyethyl) trimethyl-C_1^{14}-ammonium chloride	4	–	–	10.9	–	–
Norleucine-3-C^{14} hydrochloride	2	0.6	10	17.4	2.91	10.6
Valine-4, 4'-C_2^{14} hydrochloride	1	0.3	9	8.2	0.92	14
Cholesterol-4-C^{14} (under vacuum)	0	–	–	–	1.48	2.3

It is quite apparent that preparations of high specific activity that have been stored for any period of time must be examined for deterioration prior to use. The most convenient general method of detecting radio-contaminants is by filter-paper partition chromatography and subsequent radioautography. Volatile components must be trapped and converted to suitable, less volatile derivatives.[109,148]

Four methods of providing a radiation absorber to minimize autodecomposition have been proposed.[110,145] These methods are self-dilution (i.e., dilution with the nonlabeled material), solution dilution with a readily separable solvent[141, 143, 144] (simple aromatic hydrocarbons being the most stable known), solid dilution by dispersal as a finely divided solid in a readily separable inert material such as powdered glass, and dispersal as a film, one or more orders of magnitude thinner than an "infinitely thick" layer.

G. OTHER METHODS OF PREPARING LABELED ORGANIC COMPOUNDS

Although the use of chemical synthetic methods is generally advisable for the preparation of labeled compounds, there are many materials of biological interest which may not be expediently synthesized by the customary methods of organic chemistry. Living organisms utilizing labeled substrates have been widely employed in these cases. Such methods have not been included in this book; considerations about biosyntheses of labeled compounds have been presented by various authors,[151, 152] and a bibliography with 345 references has been published.[153]

In addition to the usual procedures for preparing labeled compounds from labeled precursors, approaches based on the momentum properties of nuclei appear to offer convenient and generally useful methods for the direct labeling of organic compounds. When a nuclear transformation occurs, the resulting species has a recoil momentum which is very large in chemical terms. Such recoil or "hot" atoms can break chemical bonds by collision with other atoms they encounter, and may then combine chemically with a fragment thus produced. The recoil behavior of radioactive halogen atoms following neutron capture in organic solutions has been extensively investigated.[154]

The irradiation of nitrogen-containing organic compounds or mixtures with thermal neutrons has resulted in C^{14}-labeled materials through incorporation of the recoil C^{14}-atoms produced in the $N^{14}(n,p)C^{14}$ reaction.[155-161]

The technique appears to be more applicable to the preparation of tritium-labeled compounds using recoil tritons from the $Li^6(n,\alpha)H_3$ reaction; a mixture of the organic compound and a lithium salt is irradiated

in a thermal neutron flux.[162-165] Since only one bond need be broken in this process, less disruption of the skeleton is required than with recoil C^{14}-atoms.

A somewhat analagous method has been devised in which tritium ions (from tritium gas) that have been accelerated in a simple discharge tube combine with organic material deposited on the cathode.[166] Another method not requiring the use of a nuclear reactor results in tritium labeling by "self-radiation" when an organic compound is sealed off with a small amount of essentially pure tritium gas for a period of several days.[167]

All of these direct methods are similar in that a stable atom in an organic molecule is replaced by its radioactive isotope, which is incorporated in a more or less (but not completely) random manner. Small amounts of highly active impurities are formed, which necessitates rigorous purification procedures.

H. HEALTH HAZARDS AND DISPOSAL OF RADIOACTIVE MATERIALS

1. Health Hazards

Health hazards associated with the synthesis of compounds labeled with beta-emitting isotopes are considered rather slight if the ordinary precautions of good laboratory practice are adhered to. Tritium, C^{14} and S^{35} pose no external radiation problem because of the short path and low penetrating power of their radiations, and glass containers afford adequate protection during handling. The external hazard of isotopes that emit high energy beta particles (e.g., P^{32}, E_{max} 1.72 Mev) is somewhat greater, and shielding precautions should be taken if these isotopes are handled in relatively large amounts. The shielding problem is relatively simple, however, since about 1 mm. of copper, 4 mm. of glass or 8 mm. of Lucite completely stop beta rays of 2 Mev maximum energy.[168] The handling problem is taken care of adequately by use of tongs or secondary containers made of the appropriate thickness of Lucite.

All pure beta-emitting isotopes and their compounds are of more consequence as internal than as external hazards since they may enter the body by inhalation, ingestion or skin absorption, and irradiate the tissues and organs in which they concentrate.

Estimation of maximum permissible levels of isotopes deposited internally is an extremely complex problem and is predicated on the philosophy that no organ or tissue in which they deposit shall receive a biological dose rate greater than that produced by 300 milliroentgens per week of hard X or gamma radiation. Specific data pertaining to the maximum permissible amounts of radioisotopes in the total body and maximum permissible concentrations (for continuous exposure) in air and

in water are given in the Recommendations of the International[169] and National[170] Commissions on Radiological Protection.

The recommended maximum permissible levels for internally hazardous radioisotopes, although stringent, do not seriously complicate isotopic syntheses. Procedures are usually well rehearsed with inert materials prior to the actual labeling process and are frequently carried out in closed systems. Protection from the inhalation hazard of an inadvertent accident is provided by carrying out the synthesis in a well ventilated hood.

Inhalation of gaseous or particulate radioactive material resulting from a leaky system, fire or explosion is the most likely hazard during isotopic labeling procedures. Respiratory absorption of even relatively insoluble materials is quite high. For this reason, the desirability of carrying out such procedures in a hood cannot be overemphasized. Protection against the escape of air-borne beta-active materials into the laboratory is afforded by a continuously operating air monitor consisting of a large-volume, flow-type ionization chamber in conjunction with a sensitive electrometer circuit, and equipped with an alarm system.[171, 172] This device was designed primarily for tritium but will work for $C^{14}O^2$ and other gaseous and air-borne activities. Detection of beta contamination of laboratory surfaces and equipment is accomplished with any of the commercially available rate meters equipped with a thin-mica window Geiger tube.

Water-H_2^3 in either the gaseous or liquid state is readily absorbed through the skin and lungs. The rate of absorption through the skin is sufficiently high that exposure to an atmosphere containing water-H_2^3 will result in the entry into the body of an amount of tritium approximately equal to that taken in *via* the respiratory system. Because of the high absorption rate, water-H_2^3 of high specific activity should not be allowed to come in contact with the skin.

The total absorption of tritium gas is less than that of water-H_2^3 by a factor of about 10,000. It should be emphasized, however, that air-monitoring instruments do not distinguish between these two forms of tritium, and possible catalytic oxidation of tritium gas to the more dangerous oxide necessitates the assumption that the activity is always present as water.[173]

The gamma-emitting isotopes (see Table I, 1) must also be considered as external hazards because of the range of gamma rays in various media.[168, 174] The National Committee on Radiation Protection has recommended[175] a maximum permissible external dose of X or gamma rays of 0.3 roentgen per week. Recent recommendations by the National and International Commissions have modified this value by placing a limit of 5 roentgens on the integrated yearly dose to the gonads or the total body. For intermittent exposure of limited portions of the body (i.e., hands and forearms), excluding the lens of the eye and the gonads,

the maximum permissible exposure rate for X and gamma radiation is 1.5 roentgens per week.[168,169,175]

Detailed information on the maximum permissible levels of external radiation and the control of external radiation hazards is given in several Bureau of Standards Handbooks.[175,176-180] Comar[168] has given an excellent review of the health physics and radiation protection requirements associated with the handling of radioactive materials.

2. Waste Disposal

Recommendations for waste disposal practices are being developed by various subcommittees of the National Committee on Radiation Protection.[181-183] Comar[168] has considered specific details of waste disposal, and for convenience has presented disposal methods on the basis of half-life of the isotope.

Isotopes of short half-life (I^{131} and P^{32}).—Disposal into the sewer is usually most convenient. The value for maximum short-period contamination of sewage with I^{131} and P^{32} has been set at 0.1 mc. per liter.

Isotopes of intermediate half-life (S^{35}).—No recommendations have been formulated for radioisotopes in this category. If they are put in the sewer, it has been suggested that enough of the stable isotope in the same form be added to them to give a dilution such that a constant intake could not result in more than the maximum permissible body burden.

Isotopes of long half-life (C^{14} and tritium).—The tremendous dilution of C^{14} with carbon of the biosphere makes possible liberal recommendations. Carbon-14 may be disposed of in any manner, provided it is diluted to a ratio of less than 1 μc. of C^{14} to every 10 g. of stable carbon in the same chemical form. Depending upon the chemical form, in recommended dilutions, C^{14} compounds may be discharged to sewers, incinerated, discharged to the atmosphere, mixed with garbage, or buried. Tritium, as H_2^3O, is greatly diluted by water in the environment. Tritium-labeled materials in reasonable amounts may be discharged directly to the atmosphere, burned, or discharged to sewers in much the same way as C^{14}.

Cleaning of contaminated laboratory glassware (which is essentially a waste disposal problem) is best accomplished with the usual heated acid-bath confined in a well ventilated hood.

Before disposal of any radioactive wastes, methods should be checked for compliance with the regulations of appropriate local, state and licensing agencies.

REFERENCES

1. A. M. Patterson, L. T. Capell and M. A. Magill, Chem. Abstracts, 39, 5874 (1945).
2. C. J. Collins, C. E. Crompton, A. R. Ronzio and B. M. Tolbert, ORNL 1084, Oct., 1951; Nuclear Sci. Abstr., 5, 6115 (1951).

3. J. W. Otvos and C. D. Wagner, Science, *106*, 409 (1947).
4. R. C. Anderson, Brookhaven Conference Report, Chemistry Conference No. 4, January 19–20, 1950, BNL–44, pp. 110–129, Mar., 1950; Nuclear Sci. Abstr., *4*, 4855 (1950).
5. C. L. Comar, *Radioisotopes in Biology and Agriculture*, McGraw-Hill, New York, 1955, p. 201.
6. *Isotope Index*, Vol. I, Scientific Equipment Corp., Indianapolis, Ind., June, 1956.
7. *Catalog and Price List of Radioisotopes, Special Materials and Services*, Oak Ridge National Laboratory, Oak Ridge, Tenn.
8. M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949.
9. N. D. Cheronis, *et al.*, "Micro and Semimicro Methods," in A. Weissberger, ed., *Technique of Organic Chemistry*, Vol. VI, Interscience, New York, 1954.
10. R. P. Linstead, J. A. Elvidge and M. Whalley, *A Course in Modern Techniques of Organic Chemistry*, Butterworths Scientific Publications, London, 1955.
11. R. F. Glascock, *Isotopic Gas Analysis for Biochemists*, Academic Press Inc., New York, 1954.
12. A. Guthrie and R. K. Wakerling, *Vacuum Equipment and Techniques*, McGraw-Hill, New York, 1949.
13. R. T. Sanderson, *Vacuum Manipulation of Volatile Compounds*, Wiley, New York, 1948.
14. J. Yarwood, *High Vacuum Technique*, 2nd ed., Wiley, New York, 1945.
15. A. L. Reimann, *Vacuum Technique*, Chapman and Hall Ltd., London, 1952.
16. G. Mönch, *Vakuumtechnik im Laboratorium*, Wagner, Weimar, 1937; Edwards Brothers, Ann Arbor, 1944.
17. S. Dushman, *Vacuum Technique*, Wiley, New York, 1949.
18. S. Jnanananda, *High Vacua*, D. Van Nostrand Company, New York, 1947.
19. G. K. Schweitzer and I. B. Whitney, *Radioactive Tracer Techniques*, D. Van Nostrand Company, New York, 1949.
20. S. L. Jones, I. D. Robinson, B. H. Arison and N. R. Trenner, Anal. Chem., *28*, 482 (1956).
21. N. R. Trenner, B. H. Arison and R. W. Walker, Anal. Chem., *28*, 530 (1956).
22. A. S. Keston, D. Rittenberg and R. Schonheimer, J. Biol. Chem., *122*, 227 (1937).
23. R. N. Jones and C. Sandorfy in *Chemical Applications of Spectroscopy*, W. West, ed., Interscience, New York, 1956, p. 406.
24. H. P. Broida, H. J. Morowitz and M. Selgin, J. Research Natl. Bur. Standards, *52*, 293 (1954).
25. H. B. Barbour and W. F. Hamilton, Am. J. Physiol., *69*, 654 (1924).
26. G. Popják, Biochem. J., *46*, 558 (1950).
27. J. Graf and D. Rittenberg, Anal. Chem., *24*, 878 (1952).
28. C. A. Dubbs, Anal. Chem., *25*, 828 (1953).
29. M. Orchin, I. Wender and R. A. Friedel, Anal. Chem., *21*, 1072 (1949).
30. L. Friedman and A. P. Irsa, Anal. Chem., *24*, 876 (1952).
31. A. V. Grosse, S. G. Hindin and A. D. Kirshenbaum, Anal. Chem., *21*, 387 (1949).
32. R. N. Boos, S. L. Jones and N. R. Trenner, Anal. Chem., *28*, 391 (1956).
33. J. C. Kluyver, Rec. trav. chim., *74*, 322 (1955).
34. R. E. Ferguson and H. P. Broida, Anal. Chem., *28*, 1436 (1956).
35. P. F. Holt and B. P. Hughes, J. Chem. Soc., *1953*, 1666.

36. S. L. Jones and N. R. Trenner, Anal. Chem., *28*, 387 (1956).
37. D. Rittenberg in *Preparation and Measurement of Isotopic Tracers,* E. W. Wilson, A. O. C. Nier and S. P. Reimann, eds., J. W. Edwards, Ann Arbor, Mich., 1946, p. 31.
38. P. F. Holt and B. I. Hopson-Hill, J. Chem. Soc., *1952*, 4251.
39. M. Hoch and H. Weisser, Helv. Chim. Acta, *33*, 2128 (1950).
40. H. Taube in Ann. Rev. Nuclear Sci., *VI*, 277 (1956).
41. M. Cohn and H. C. Urey, J. Am. Chem. Soc., *60*, 679 (1938).
42. I. Dostrovsky and F. S. Klein, Anal. Chem., *24*, 414 (1952).
43. V. A. Aluise, Anal. Chem., *23*, 530 (1951).
44. W. von E. Doering and E. Dorfman, J. Am. Chem. Soc., *75*, 5595 (1953).
45. M. L. Bender and R. S. Dewey, J. Am. Chem. Soc., *78*, 317 (1956).
46. M. Anbar, I. Dostrovsky, F. S. Klein and D. Samuel, J. Chem. Soc., *1955*, 155.
47. M. L. Eidinoff and J. E. Knoll, Science, *112*, 250 (1950).
48. F. N. Hayes and R. G. Gould, Science, *117*, 480 (1953).
49. D. J. Rosenthal and H. O. Anger, Rev. Sci. Instr., *25*, 670 (1954).
50. N. Pace, L. Kline, H. K. Schachman and M. Harfenist, J. Biol. Chem., *168*, 459 (1947).
51. W. H. Langham, W. J. Eversole, F. N. Hayes and T. T. Trujillo, J. Lab. Clin. Med., *47*, 819 (1956).
52. K. E. Wilzbach, L. Kaplan and W. G. Brown, Science, *118*, 522 (1953).
53. F. C. Henriques, Jr. and C. Margnetti, Ind. Eng. Chem., Anal. Ed., *18*, 420 (1946).
54. C. G. Swain, V. P. Kreiter and W. A. Sheppard, Anal. Chem., *27*, 1157 (1955).
55. M. W. Biggs, D. Kritchevsky and M. R. Kirk, Anal. Chem., *24*, 223 (1952).
56. H. von Buttlar and W. F. Libby, J. Inorg. and Nuclear Chem., *1*, 75 (1955).
57. W. Bernstein and R. Ballentine, Rev. Sci. Instr., *21*, 158 (1950).
58. D. F. White, I. G. Campbell and P. R. Payne, Nature, *166*, 628 (1950).
59. C. V. Robinson, Nucleonics, *13* (11), 90 (1955).
60. R. F. Glascock, *Isotopic Gas Analysis for Biochemists,* Academic Press Inc., New York, 1954, p. 180.
61 A. Schwebel, H. S. Isbell and J. V. Karabinos, Science, *113*, 465 (1951).
62. D. L. Tabern and T. N. Lahr, Science, *119*, 739 (1954).
63. C. Entenman, S. R. Lerner, I. L. Chaikoff and W. G. Dauben, Proc. Soc. Exptl. Biol. Med., *70*, 364 (1949).
64. J. R. Hogness, L. J. Roth, E. Leifer and W. H. Langham, J. Am. Chem. Soc., *70*, 3840 (1948).
65. J. C. Roucayrol and E. Oberhausen, Science, *122*, 201 (1955).
66. F. N. Hayes, R. D. Hiebert and R. L. Schuch, Science, *116*, 140 (1952).
67. E. C. Farmer and I. A. Berstein, Science, *115*, 460 (1952).
68. M. S. Raben and N. Bloembergen, Science, *114*, 363 (1951).
69. F. N. Hayes, B. S. Rogers and W. H. Langham, Nucleonics, *14* (3), 48 (1956).
70. C. C. White and S. Helf, Nucleonics, *14* (10), 46 (1956).
71. B. L. Funt, Nucleonics, *14* (8), 83 (1956).
72. F. C. Henriques, Jr. and C. Margnetti, Ind. Eng. Chem., Anal. Ed., *18*, 417 (1946).
73. V. F. Raaen and G. A. Ropp, Anal. Chem., *25*, 174 (1953).
74. G. L. Brownell and H. S. Lockhart, Nucleonics, *10* (2), 26 (1952).
75. G. J. Fergusson, Nucleonics, *13* (1), 18 (1955).
76. S. C. Brown and W. W. Miller, Rev. Sci. Instr., *18*, 496 (1947).

77. D. R. Christman, N. E. Day, P. R. Hansell and R. C. Anderson, Anal. Chem., *27*, 1935 (1955).
78. D. R. Christman and A. P. Wolf, Anal. Chem., *27*, 1939 (1955).
79. W. F. Libby, *Radiocarbon Dating*, 2nd ed., University of Chicago Press, Chicago, Ill., 1955.
80. W. H. Burke and W. G. Meinschein, Rev. Sci. Instr., *26*, 1137 (1955).
81. R. W. Pringle, W. Turchinetz and B. L. Funt, Rev. Sci. Instr., *26*, 859 (1955).
82. H. E. Suess, Science, *120*, 5 (1954).
83. J. M. Passman, N. S. Radin and J. A. D. Cooper, Anal. Chem., *28*, 484 (1956).
84. F. N. Hayes, E. C. Anderson and J. R. Arnold, Proc. Intern. Conf. Peaceful Uses Atomic Energy, Geneva, *14*, 188 (1956).
85. J. R. Arnold, Science, *119*, 155 (1954).
86. L. R. Wasserman, T. F. Yoh and I. A. Rashkoff, J. Lab. Clin. Med., *37*, 342 (1951).
87. R. Loevinger and S. Feitelberg, Nucleonics, *13* (4) 42 (1955).
88. M. Walser, A. F. Reid and D. W. Seldin, Arch, Biochem. Biophys., *45*, 91 (1953).
89. W. L. Graf, S. L. Comar and I. B. Whitney, Nucleonics, *9* (4), 22 (1951).
90. L. Young, M. Edson and J. A. McCarter, Biochem. J., *44*, 179 (1949).
91. E. C. Henriques, Jr., G. B. Kistiakowsky, G. Margnetti and W. G. Schneider, Ind. Eng. Chem., Anal. Ed., *18*, 349 (1946).
92. M. Kahn, A. J. Freedman, R. D. Feltham and N. L. Lark, Nucleonics, *13* (5), 58 (1955).
93. J. H. Weisburger and H. J. Lipner, Nucleonics, *12* (5), 21 (1954).
94. J. E. Francis, P. R. Bell and C. C. Harris, Nucleonics, *13* (11), 82 (1955).
95. G. J. Hine, B. A. Burrows, L. Apt, M. Pollycove, J. F. Ross and L. A. Sarkes, Nucleonics, *13* (2), 23 (1955).
96. F. N. Hayes, B. S. Rogers and P. C. Sanders, Nucleonics, *13* (1) 46 (1955).
97. F. N. Hayes, D. G. Ott, V. N. Kerr and B. S. Rogers, Nucleonics, *13* (12), 38 (1955).
98. F. N. Hayes, D. G. Ott and V. N. Kerr, Nucleonics, *14* (1), 42 (1956).
99. F. N. Hayes, J. Appl. Rad. Isotopes, *1*, 46 (1956).
100. F. N. Hayes and D. G. Ott, Atomic Energy Commission Report, LA-2095.
101. V. N. Kerr, F. N. Hayes and D. G. Ott, J. Appl. Rad. Isotopes, *1*, 284 (1957).
102. D. L. Williams, F. N. Hayes, R. J. Kandel and W. H. Rogers, Nucleonics, *14* (1), 62 (1956).
103. M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, Chapter 10.
104. A. A. Benson, J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas and W. Stepka, J. Am. Chem. Soc., *72*, 1710 (1950).
105. M. Calvin and A. A. Benson, Science, *109*, 140 (1949).
106. R. Consden, A. H. Gordon and A. J. P. Martin, Biochem. J., *38*, 224 (1944).
107. S. M. Partridge, Biochem. J., *42*, 238 (1948); Nature, *158*, 270 (1946).
108. S. Aronoff, *Techniques of Radiobiochemistry*, The Iowa State College Press, Ames, Iowa, 1956, Chapters 2 and 3.
109. B. M. Tolbert, P. T. Adams, E. L. Bennett, A. M. Hughes, M. R. Kirk, R. M. Lemmon, R. M. Noller, R. Ostwald and M. Calvin, J. Am. Chem. Soc., *75*, 1867 (1953).
110. R. M. Lemmon, Nucleonics, *11* (10), 44 (1953).

111. R. J. Block, E. L. Durrum and G. Zweig, *Paper Chromatography and Paper Electrophoresis,* Academic Press, New York, 1954.
112. F. Cramer, *Paper Chromatography,* 2nd ed., St. Martin's Press, New York, 1954.
113. R. Kunin and R. J. Meyers, *Ion Exchange Resins,* Wiley, New York, 1950.
114. F. C. Nachod, *Ion Exchange,* Academic Press, New York, 1949.
115. Williams, *The Elements of Chromatography,* Blackie and Son, London, 1954.
116. J. N. Balston and B. E. Talbot, *A Guide to Filter Paper and Cellulose Powder Chromatography,* H. R. Angel and Co., Ltd., London, and W. and R. Balston, Ltd., Maidstone, England, 1952.
117. R. C. Brimley and F. C. Barrett, *Practical Chromatography*, Reinhold, New York, 1953.
118. E. Lederer and M. Lederer, *Chromatography,* Elsevier, New York, 1954.
119. L. Zechmeister, *Progress in Chromatography* 1938–1947, Wiley, New York, 1950.
120. L. Zechmeister and L. Cholnoky, *Principles and Practice of Chromatography*, Wiley, New York, 1951.
121. R. L. Block, *Paper Chromatography* (A Laboratory Manual), Academic Press, New York, 1952.
122. C. Phillips, *Gas Chromatography*, Butterworths Scientific Publications, London, 1956.
123. H. G. Cassidy, "Adsorption and Chromatography," *Technique of Organic Chemistry,* Vol. V, A. Weissberger, ed., Interscience, New York, 1951.
124. A. Tiselius, Nord. Kemistmötet, 7, 70 (1950).
125. R. J. Dimler, Trans. Am. Assoc. Cereal Chemists, *12*, 1 (1954).
126. G. Hesse, Angew. Chem., *67*, 9 (1955).
127. W. Buser, P. Graf and W. F. Grütter, (346 references), Chimia (Switz.), *9*, 73 (1955).
128. J. F. Duncan and B. A. J. Lister, Quart Rev. Chem. Soc., *2*, 307 (1948).
129. S. Moore and W. H. Stein, Ann. Rev. Biochem., *21*, 521 (1952).
130. E. L. Streatfield, Chem. and Ind. (London), *1953*, 1214.
131. J. D. Devey, *Paper Chromatography*, (a selective bibliography of 1085 references), Research Equipment Corp., 1135 Third Street, Oakland, California.
132. "Symposium on Chromatographic Analysis," Discussions Faraday Soc., 7, 1–336 (1949).
133. "Symposium on Chromatography," Brit. Med. Bull., *10* (3), 161–250 (1954).
134. G. A. Boyd, *Autoradiography in Biology and Medicine,* Academic Press, New York, 1955.
135. G. A. Ropp, Nucleonics, *10* (10), 22 (1952).
136. P. E. Yankwich, "Isotope Effects in Chemical Reactions," Ann. Rev. Nuclear Sci., *3*, 235–248 (1953).
137. K. B. Wiberg, Chem. Revs., *55* (4), 713 (1955).
138. A. L. Promislow, Univ. Microfilms (Ann Arbor, Mich.), Publ. No. 11,527; Chem. Abstracts, *49*, 11334 (1955).
139. S. Aronoff, *Techniques of Radiobiochemistry*, Iowa State College Press, Ames, Iowa, 1956, p. 6.
140. C. L. Comar, *Radioisotopes in Biology and Agriculture,* McGraw, New York, 1955, p. 76.
141. B. M. Tolbert and R. M. Lemmon, Atomic Energy Commission Report, UCRL–2704; Radiation Research, 3, 52 (1055); Nuclear Sci. Abstr. *9*, 102 (1955).
142. E. Collinson and A. J. Swallow, Chem. Revs., *56*, 471 (1956); Quart. Revs. (London), *9*, 311 (1955).

143. *Radiation Chemistry*, Discussions Faraday Soc., *12*, 13 (1952).
144. M. Burton, J. Phys. and Colloid Chem., *52*, 564 (1948).
145. C. D. Wagner and V. P. Guinn, J. Am. Chem. Soc., *75*, 4861 (1953).
146. W. J. Skraba, J. G. Burr, Jr. and D. N. Hess, J. Chem. Phys., *21*, 1296 (1953).
147. W. G. Dauben and P. H. Payot, J. Am. Chem. Soc., *78*, 5657 (1956).
148. R. M. Lemmon, M. A. Parsons and D. M. Chin, J. Am. Chem. Soc., *77*, 4139 (1955).
149. D. L. Williams and A. R. Ronzio, Atomic Energy Commission Report, AECU-2126; Nuclear Sci. Abstr., *6*, 5075 (1952).
150. P. T. Adams, J. Am. Chem. Soc., *77*, 5357 (1955).
151. M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, Chapter 12.
152. Abstracts of a Conference on The Use of Isotopes in Plant and Animal Research, June 1952, Agricultural Experiment Station Report No. 4, Kansas State College. (Available from Supt. of Documents, U. S. Gov't. Printing Office, Washington 25, D. C. as TID-5098, April 1953.)
153. "Selected Reference Material on Atomic Energy, Vol. VII, Eight-Year Isotope Summary," International Conference on Peaceful Uses of Atomic Energy at Geneva, August 1955. (Available from Supt. of Documents, U. S. Gov't. Printing Office, Washington 25, D. C.).
154. J. Willard, Ann. Review Nuclear Sci., *3*, 193 (1953); Ann. Rev. Phys. Chem., *6*, 141 (1955).
155. G. Giacomello, Ricerca sci., *21*, 1211 (1951).
156. U. Croatto, G. Giacomello, and A. G. Maddock, Ricerca sci., *21*, 1598 (1951).
157. A. G. Schrodt and W. F. Libby, J. Am. Chem. Soc., *76*, 3100 (1954).
158. A. P. Wolf and R. C. Anderson, J. Am. Chem. Soc., *77*, 1608 (1955).
159. P. E. Yankwich and W. R. Cornman, Jr., J. Am. Chem. Soc., *78*, 1560 (1956).
160. A. P. Wolf, B. Gordon and R. C. Anderson, J. Am. Chem. Soc., *78*, 2657 (1956).
161. R. Wolfgang, R. C. Anderson and R. W. Dodson, J. Chem. Phys., *24*, 16 (1956).
162. R. Wolfgang, F. S. Rowland and C. N. Turton, Science, *121*, 715 (1955).
163. F. S. Rowland, C. N. Turton and R. Wolfgang, J. Am. Chem. Soc., *78*, 2354 (1956).
164. R. Wolfgang, J. Eigner and F. S. Rowland, J. Phys. Chem., *60*, 1137 (1956).
165. M. Tsutsui, H. L. Bradlow and D. K. Fukushima, Abst. of 129th Meeting of the Am. Chem. Soc., Dallas, Tex., April 1956, p. 31N.
166. R. Wolfgang, T. Pratt and F. S. Rowland, J. Am. Chem. Soc., *78*, 5132 (1956).
167. K. E. Wilzbach, J. Am. Chem. Soc., *79*, 1013 (1957).
168. C. L. Comar, *Radioisotopes in Biology and Agriculture*, McGraw-Hill, New York, 1955.
169. "Recommendations of the International Commission on Radiological Protection and of the International Commission on Radiological Units (1950)," Natl. Bur. Standards Handbook 47, June 29, 1951.
170. "Maximum Permissible Amounts of Radioisotopes in the Human Body and Maximum Permissible Concentrations in Air and Water," Natl. Bur. Standards Handbook 52, Mar. 20, 1953.
171. B. C. Eutsler, G. L. Evans, R. D. Hiebert, R. N. Mitchell, M. C. Robbins and R. J. Watts, Nucleonics, *14* (9), 114 (1956).

172. C. D. Yaffe, D. H. Byers and H. D. Hosey, *Encyclopedia of Instruments for Industrial Hygiene,* Univ. of Mich. Press, Ann Arbor, 1956.
173. E. A. Pinson and W. H. Langham, J. Appl. Physiol., *10*, 108, (1957).
174. "Safe Handling of Radioactive Isotopes," Natl. Bur. Standards Handbook 42, Sept. 1949.
175. "Permissible Dose from External Sources of Ionizing Radiation," Natl. Bur. Standards Handbook 59, Sept. 24, 1954.
176. "Radiological Monitering Methods and Instruments," Natl. Bur. Standards Handbook 51, April 7, 1952.
177. "Medical X-Ray Protection up to Two Million Volts," Natl. Bur. Standards Handbook 41, Mar. 30, 1949.
178. "X-Ray Protection Design," Natl. Bur. Standards Handbook 50, May 9, 1952.
179. "Protection against Radiations from Radium, Cobalt-60 and Cesium-137," Natl. Bur. Standards Handbook 54, Sept. 1, 1954.
180. "X-Ray Protection," Natl. Bur. Standards Handbook 60, Dec. 1, 1955.
181. "Recommendations for Waste Disposal of Phosphorus-32 and Iodine-131 for Medical Users," Natl. Bur. Standards Handbook 49, Nov. 2, 1951.
182. "Recommendations for the Disposal of Carbon-14 Wastes," Natl. Bur. Standards Handbook 53, Oct. 26, 1953.
183. "Control and Removal of Radioactive Contamination in Laboratories," Natl. Bur. Standards Handbook 48, Dec. 15, 1951.

ACIDS

SODIUM FORMATE-C^{14}

METHOD I

$$KC^*N \xrightarrow{H_2O} HC^*OOK$$

D. G. Grant and H. S. Turner, Nature, *165*, 153 (1950).

A. Procedure (Note 1)

An aqueous solution of carbonate-free potassium cyanide-C^{14} (Note 2), in a stainless steel bomb (Note 3), is shock-heated (to minimize the possibility of polymerization) to 150° and maintained at that temperature for 4 hours (Note 4). The cooled solution is acidified with excess phosphoric acid and distilled at room temperature in a closed system under high vacuum. Sodium formate-C^{14} is isolated in 98–100% over-all yield by neutralization and evaporation of the distillate (Note 5).

METHOD II

$$NaHC^*O_3 \xrightarrow[Pd]{H_2} HC^*OONa$$

J. W. Spyker and A. C. Neish, Can. J. Chem., *30*, 461 (1952).

A. Procedure (Note 6)

A solution of sodium carbonate-C^{14} in 0.5*N* sodium hydroxide is treated with sufficient sodium bicarbonate to give 2 mmoles, and then is titrated with formic acid to the bicarbonate end point, with use of meta-cresol purple indicator (Note 7). The solution is transferred into a glass bomb liner, with sufficient water to make 20 ml., then treated with 2 ml. of a 6% suspension of palladium-black (Note 8) and hydrogenated at 3700 p.s.i. for 24 hours at 80°. The catalyst is filtered out, the filtrate is acidified with formic acid (Note 9), and the carbon dioxide is swept out by carbon dioxide-free air. The solution is titrated to the phenol red

end point with sodium hydroxide (Note 10) and decolorized with charcoal; then the filtrate is evaporated to dryness on a steam-bath; yield 95-97% (3 runs).

B. Notes

1. The procedure is essentially the isotopic one described by Heidelberger.[1]

2. All concentrations of solution up to saturation at room temperature may be used. The presence of carbonate does not affect the yield of formate, but there is an isotopic exchange. When a mixture of potassium cyanide and potassium carbonate-C^{14} (20% by weight) is hydrolyzed under these conditions, the specific activity of the carbonate-C^{14} falls to 74% of the initial value. When the carbonate is precipitated by excess baryta, prior to hydrolysis, but is left in the solution, the activity falls to 90%.

3. A specially designed small autoclave, described by Kantorowicz,[2] is heated with an oil-bath.

4. The hydrolysis may be effected by heating at 175° for 2 hours; Arnstein[3] heats for 2 hours at 250°.

5. Arnstein[3] collected the product beneath an excess of sodium hydroxide solution, by steam-distillation from an excess of dilute sulfuric acid. The excess alkali is neutralized with formic acid. A micro method for determining formic acid is described by Weygand.[4]

6. The procedure is a simplification of the isotopic procedure described by Melville.[5]

7. Kögl[6] has described the formation of the bicarbonate-C^{14} by titration of a sodium hydroxide solution of the carbonate-C^{14} with 0.1*N* sulfuric acid to the phenolphthalein endpoint, thereby avoiding isotopic dilution. A diagram of an all-glass system for a micro preparation of formic-C^{14} acid by this method is given.[6] du Vigneaud[7] has employed hydrochloric acid for this titration.

The preparation of sodium bicarbonate-C^{14} solution by absorption of carbon-C^{14} dioxide in the calculated amount of carbonate-free 1 *N* sodium hydroxide has been described by Allen[8] and Kaltenbach,[9] see Figure II, 1.

8. Palladium chloride is reduced with alkaline formaldehyde at 4-8°.

9. Isotopic dilution may be avoided by isolation of the product as described in Method I, or with Dowex-50 cation exchange resin.[10] Steam distillation gives a very pure product.

10. Formic acid may be determined by the reduction of mercuric chloride.[11]

C. Other Preparations

Sodium formate-C^{13} has been prepared by Arnstein and Bentley,[3] by essentially the procedure described in Method I.

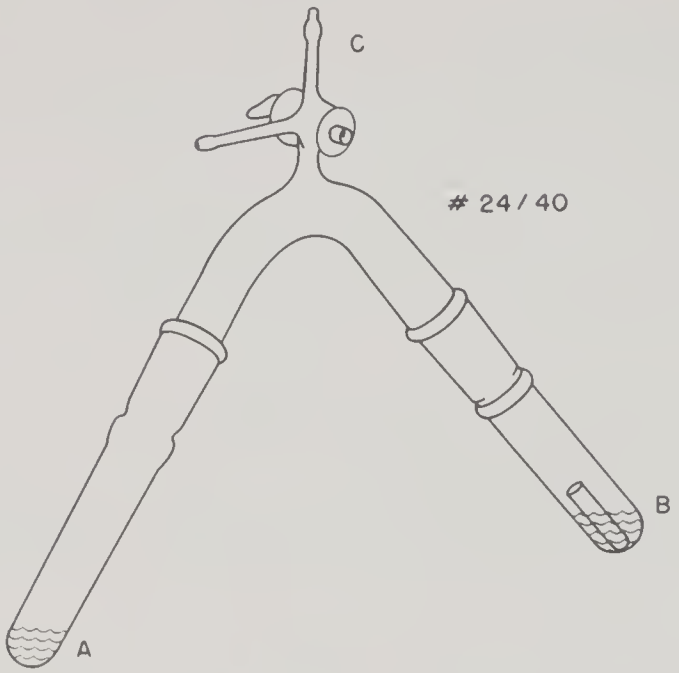


Fig. II, 1. Apparatus for the preparation of bicarbonate (M. B. Allen, H. Gest and M. D. Kamen). A, Alkali; B, acid vial; C, outlet to vacuum.

Sodium formate-C^{13} has been prepared in nearly quantitative yield by Sprinson,[12] by the hydrolysis of cyanide-C^{13} in 0.5 *N* sodium hydroxide at 175° for 5 hours.

The preparation of labeled formic acid by the acid hydrolysis of isotopic cyanide in a sealed tube has been reported by Sonne,[13] Heinrich[14] and by van den Bos;[15] yields[16] 50–60%.

The preparation of formate-C^{14} by hydrogenation of bicarbonate-C^{14}, essentially by the procedure of Method II, has been described by a number of investigators. Melville[5] (98–99%), Weygand[4] (90–95%), Adams[10] (85–90%), Dische[17] (practically quantitative), and Totter[18] have employed the potassium salt; while Kögl[6] (90–95%), du Vigneaud,[7] Berg,[19] Freudenberg[20a] (3 days, 70°, 120 atm.) and Wilson[20] have employed the sodium salt. Pichat[21] obtained his best yield (97%) with the potassium salt, after adjusting the bicarbonate solution to pH 8.2 with 0.1 *N* sulfuric acid. Anker[22] (70%) found no difference due to salts, but attributed the low yield to the use of commercial catalyst.

The preparation of sodium formate-C^{14} by the reduction of carbon-C^{14} dioxide (75%, average of 5 runs, based on unrecovered carbon-C^{14} dioxide) with lithium borohydride in ether solution at 0° has been described by Burr,[23] with diagrams of several systems given. A by-product is methanol-C^{14} (10–15%) along with unreacted carbon-C^{14} dioxide (15–20%).

Sodium formate-C^{14} has been prepared by Murray[24] (97.7–99.5% yield) by oxidation of formaldehyde-C^{14} with 20% hydrogen peroxide after the method of Blank and Finkenbeiner.[25]

The reduction of carbon-C^{11} dioxide to formic-C^{11} acid by the action of a suspension of *Bacterium coli* has been reported by Harman.[26]

[1]C. Heidelberger and R. Hurlbert, Nucleonics, 7 (3), 54 (1950); H. Sulzer, Angew. Chem., *25*, 1268 (1912).
[2]O. Kantorowicz, J. Soc. Chem. Ind. (London), *69*, Suppl. No. 2, S76 (1950).
[3]H. R. V. Arnstein and R. Bentley, J. Chem. Soc., *1951*, 3509; H. R. V. Arnstein, Biochem. J., *48*, 27 (1951).
[4]F. Weygand and O. A. Grosskinsky, Ber., *84*, 839 (1951).
[5]D. B. Melville, J. R. Rachele and E. B. Keller, J. Biol. Chem., *169*, 419 (1947); G. Bredig and S. R. Carter, Ber., *47*, 541 (1914).
[6]F. Kögl, J. Halberstadt and T. J. Barendregt, Rec. trav. chim., *68*, 387 (1949).
[7]V. du Vigneaud, W. G. L. Verly, J. E. Wilson, J. R. Rachele, C. Ressler and J. M. Kinney, J. Am. Chem. Soc., *73*, 2782 (1951).
[8]M. B. Allen, H. Gest and M. D. Kamen, Arch. Biochem., *14*, 335 (1947); J. M. Siegel, J. Biol. Chem., *208*, 205 (1954).
[9]J. P. Kaltenbach and G. Kalnitsky, J. Biol. Chem., *192*, 629 (1951).
[10]P. T. Adams, R. E. Selff and B. M. Tolbert, J. Am. Chem. Soc., *74*, 2416 (1952); Atomic Energy Commission Report, UCRL-1289; Nuc. Sci. Abstracts, *5*, 4405 (1951).
[11]E. M. Benedict and G. A. Harrop, J. Biol. Chem., *54*, 443 (1922).
[12]D. B. Sprinson and D. Rittenberg, J. Biol. Chem., *198*, 655 (1952).
[13]J. C. Sonne, J. M. Buchanan and A. M. Delluva, J. Biol. Chem., *173*, 69 (1948).
[14]M. R. Heinrich and D. W. Wilson, J. Biol. Chem., *186*, 447 (1950).
[15]B. G. van den Bos and A. H. W. Aten, Jr., Rec. trav. chim., *70*, 495 (1951).
[16]M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon,* Wiley, New York, 1949, p. 165.
[17]R. Dische and D. Rittenberg, J. Biol. Chem., *211*, 199 (1954).
[18]J. R. Totter, J. Am. Chem. Soc., *76*, 2196 (1954).
[19]P. Berg, J. Biol. Chem., *205*, 145 (1953).
[20]D. G. Wilson, K. W. King and R. H. Burris, J. Biol. Chem., *208*, 863 (1954).
[20a]K. Freudenberg and W. Fuchs, Ber., *87*, 1824 (1954).
[21]L. Pichat, M. Audinot and J. Monnet, Bull. soc. chim. France, (5) *21*, 85 (1954).
[22]R. Anker, Nucleonics, 7 (3), 54 (1950).
[23]J. G. Burr, Jr., W. G. Brown and H. E. Heller, J. Am. Chem. Soc., *72*, 2560 (1950).
[24]A. Murray III, C. W. Bills and A. R. Ronzio, J. Am. Chem. Soc., *74*, 2405 (1952).
[25]O. Blank and H. Finkenbeiner, Ber., *31*, 2979 (1898).
[26]D. Harman, T. D. Stewart and S. Ruben, J. Am. Chem. Soc., *64*, 2293 (1942).

SODIUM ACETATE-1-C^{14}

METHOD I

$$CH_3MgI \xrightarrow{C^*O_2} CH_3C^*OOMgI \xrightarrow{H^+} CH_3C^*OOH \xrightarrow{NaOH} CH_3C^*OONa$$

A. Murray III, Los Alamos Scientific Laboratory, unpublished work.

A. Procedure (Note 1)

A solution of 13.28 mmoles of methylmagnesium iodide in 42 ml. of ether, under dry nitrogen, is frozen with liquid nitrogen in the flask of

a Grignard carbonation apparatus (Note 2). The entrapped nitrogen is removed by several cycles of thawing at −80°, refreezing, and evacuation to 0.1 μ. The dry carbon-C^{14} dioxide from 0.6555 g. (3.32 mmoles) of barium carbonate-C^{14} is distilled into the flask with liquid nitrogen. The flask is isolated from the manifold and immersed in a Dry Ice-acetone bath for 5 minutes, thawing taking place at 3 minutes and the mixture then being stirred. The flask is immersed in a bath at −20° to −25°, and the mixture is stirred for 15 minutes. Nitrogen is admitted, the bath is removed and the cold, stirred mixture is treated with 20 ml. of water, followed by 10 ml. of 20% sodium hydroxide. The ether is decanted and the aqueous slurry is transferred into a 100-ml. two-necked, round-bottomed flask containing 2.72 g. of silver sulfate (30% excess), by washing with 30 ml. of water. The residual ether and about 15 ml. of water are distilled (Note 3). The mixture is acidified with 10 ml. of 50% sulfuric acid, added through an addition funnel. The product is steam-distilled, with an oil-bath at 135–145°, into an ice-cooled receiver. Approximately 125 ml. of distillate is collected in about 2 hours by adding water to the still pot at about the same rate as it is distilled (Note 4). The distillate is titrated with standard sodium hydroxide (33.35 ml., 0.0998 *N*); yield quantitative (Note 5). The filtered solution is evaporated and dried under vacuum.

The anhydrous free acid may be prepared [1, 2, 3, 4] by heating the dry salt with gaseous hydrogen chloride in a gas-solid reactor (see chloroacetic-1-C^{14} acid). The liberated acid is collected in a trap cooled with liquid nitrogen, then pumped on at the temperature of a Dry Ice-bath to remove hydrogen chloride (Note 6).

METHOD II

$$CH_3I \xrightarrow{KC*N} CH_3C*N \xrightarrow{NaOH} CH_3C*OONa$$

J. D. Cox, H. S. Turner and R. J. Warne, J. Chem. Soc., *1950*, 3167.

A. Procedure

The procedure is similar to that described under sodium acetate-2-C^{14}, Method II, with the following modifications. A 50% excess of methyl iodide is employed. Following hydrolysis of acetonitrile-1-C^{13}, the alkaline solution is steam-distilled for 15 minutes to remove methanol; otherwise, up to 50% of the acetic-1-C^{13} acid distills as the ester. The amount of silver sulfate used is 0.4 times the number of millimoles of cyanide. The chemical and radiochemical yield (8 experiments) is 97–100%. The purity is comparable to that found with sodium acetate-2-C^{14}. The method has been used at the 3 to 250-mmole scale.

B. Notes

1. The procedure is a modification of the method of Lemmon.[5]

2. For a description of the apparatus, see α-(*p*-hydroxyphenyl)-*o*-toluic-C^{14} acid and benzoic-C^{14} acid.

3. The distillate is nonradioactive. Heating serves to concentrate the mixture and to conglomerate the silver halide.

4. The volume of distillate required to collect all the product is reduced markedly by keeping the volume of mineral acid-salt mixture small.

5. Quantitative yields have been achieved by another operator[6] at the 1.4-mmole scale. The titration is accomplished with a glass electrode, or phenolsulfonphthalein indicator. Roe and Finlay[7] have reported that the activity of a sample of dry lithium acetate-1-C^{14} did not change over a period of 8 months.

6. Hughes, Ostwald and Tolbert[1,2] have reported 98–99% yields of acetic or propionic acid, based on the salts.

C. Other Preparations

The preparation of acetic-1-C^{14} acid by the Grignard reaction has been reported by many investigators; see Table II, 1 following benzoic-C^{14} acid.

The preparation of sodium acetate-1-C^{13} by reacting sodium cyanide-C^{13} with methyl sulfate, followed by alkaline hydrolysis of the acetonitrile-1-C^{13} has been reported by Weinhouse[8] (83%), Elwyn,[9] Crandall[10] and by Bloch.[11]

Potassium acetate-2-C^{13}-1-C^{14} has been prepared by Baddiley,[12] by carbonating methylmagnesium-C^{13} iodide with carbon-C^{14} dioxide. The anhydrous salt (50 g., 51% yield) was treated with concentrated sulfuric acid, and the free acetic-2-C^{13}-1-C^{14} acid was distilled; yield 23 g. (38%), b.p. 112–119°, purity 94%.

[1]D. M. Hughes, R. Ostwald and B. M. Tolbert, J. Am. Chem. Soc., *74*, 2434 (1952).

[2]R. Ostwald, J. Biol. Chem., *173*, 207 (1948).

[3]D. Chin and P. Adams, Atomic Energy Commission Report, UCRL-2394; Nuc. Sci. Abstracts, *8*, 1057 (1954).

[4]K. Freudenberg and W. Fuchs, Ber., *87*, 1824 (1954).

[5]M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 178.

[6]D. L. Williams, unpublished work.

[7]A. Roe and J. B. Finlay, J. Am. Chem. Soc., *74*, 2442 (1952).

[8]S. Weinhouse, G. Medes and N. F. Floyd, J. Biol. Chem., *158*, 411 (1945).

[9]D. Elwyn and D. B. Sprinson, *ibid.*, *184*, 465 (1950).

[10]D. I. Crandall and S. Gurin, *ibid.*, *181*, 829 (1949).

[11]I. Zabin and K. Bloch, *ibid.*, *185*, 131 (1950); H. N. Little and K. Bloch, *ibid.*, *183*, 33 (1950).

[12]J. Baddiley, G. Ehrensvärd, R. Johansson, L. Reio, El Saluste and R. Stjernholm, *ibid.*, *183*, 771 (1950).

TABLE II, 1

Acid Synthesis by Carbonation of an Isotopic Grignard Reagent

Acid	RX	mmole	% Yield	Reference	Note
Propionic-2-C^{13}	$CH_3C^*H_2I$	—	65–80	11, 12	—
Propionic-2-C^{14}	$CH_3C^*H_2I$	—	40	13, 14	a
Propionic-2-C^{14}	$CH_3C^*H_2Br$	23	68	15, 16	b
Propionic-2-C^{14}	$CH_3C^*H_2Br$	–	65	17	–
Propionic-3-C^{13}	$C^*H_3CH_2I$	–	65–80	11	–
Propionic-3-C^{14}	$C^*H_3CH_2Br$	17.6	49.5	15	c
Butyric-2-C^{13}	$CH_3CH_2C^*H_2I$	60	22	18	d
Butyric-2-C^{14}	$CH_3CH_2C^*H_2I$	–	43	17	e
2-Methylpropionic-2-C^{14}	$(CH_3)_2C^*HBr$	–	–	19	f
2-Methylpropionic-3-C^{13}	$C^*H_3CHBrCH_3$	20	48	20	–
Octanoic-2-C^{14}	$CH_3(CH_2)_5C^*H_2Br$	–	–	21	g
Hexadecanoic-2-C^{14}	$CH_3(CH_2)_{13}C^*H_2Br$	–	–	22	h

Notes

a. The yield is based on barium carbonate-C^{14}.

b. The preparation of the Grignard reagent is described by Tolbert.[31]

c. The yield is based on sodium acetate-2-C^{14}.

d. The yield is based on barium carbonate-C^{13}. Analysis by determination of the partition coefficient showed the product to be 13.1 mmoles of butyric-2-C^{13} acid mixed with 5.3 mmoles of propionic acid. Sodium propionate-1-C^{13} was esterified with ethyl sulfate, then converted to a mixture of ethanol and 1-propanol-1-C^{13} by high-pressure hydrogenolysis. The carbinols were converted to the corresponding iodides by heating with constant-boiling hydriodic acid, and fractionated.

e. The compound is prepared in 85% yield through the nitrile.

f. 2-Bromopropane-1-C^{14} is prepared in 50% yield from 3.0 g. of acetic-1-C^{14} acid. The calcium salt is pyrolised, and the 2-propanone-2-C^{14} formed is hydrogenated over Raney nickel in purified dioxane for 5 hours at 40 p.s.i. The resulting 1-methylethanol-1-C^{14} is halogenated by treatment with phosphorus tribromide. Also see leucine-3-C^{14}.

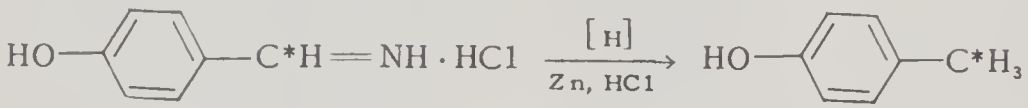
g. Heptanoic-1-C^{14} acid is reduced with lithium aluminum hydride to 1-heptanol-1-C^{14}, which is converted to 1-bromoheptane-1-C^{14} by treatment with hydrobromic and sulfuric acids, according to the method of Kamm and Marvel.[32]

h. Pentadecanoic-1-C^{14} acid is reduced with lithium aluminum hydride to 1-pentadecanol-1-C^{14} and converted to 1-bromopentadecane-1-C^{14} with hydrogen bromide; see 1-bromohexane-1-C^{14}.

SILVER ACETATE-2-C^{13}

$$NaC^*N \xrightarrow{ZnCl_2} Zn(C^*N)_2 \xrightarrow[HCl]{C_6H_5OH}$$

(a)



(b) (c)

$$\xrightarrow[2.\ Ag_2CO_3]{1.\ K_2Cr_2O_7} C^*H_3COOAg$$

(d)

H. S. Anker, J. Biol. Chem., *166*, 219 (1946).

A. Procedure

(a) *Zinc Cyanide-C^{13}*. The preparation is by a suitable adaption of the procedure of Adams and Levine,[1] which follows.

To a solution of 1.05 mmoles of crude sodium cyanide-C^{13} in 65 ml. of water is added magnesium chloride solution until no further precipitate of magnesium hydroxide or carbonate is formed. The precipitate is filtered off immediately (Note 1), and the filtrate is added at once to a saturated solution containing 1 mole of zinc chloride in 50% alcohol. The precipitated product is collected, washed with alcohol, then ether, and dried in a desiccator. The purity is about 90% (Note 2).

(b) p-*Hydroxybenzylidenimine-C^{13} Hydrochloride* (Note 3). A stirred mixture of 5.9 g. of zinc cyanide-C^{13}, 0.1 g. of potassium chloride (Note 4), 13 g. of phenol and 130 ml. of absolute ether is saturated with dry hydrogen chloride in the dark, about 1 hour being required (Note 5). The gas is introduced for 3 more hours at a reduced rate; then the precipitated product is separated by decantation and washed with three portions of absolute ether (Note 6).

(c) p-*Cresol-α-C^{13}* (Note 7). To a rapidly refluxing mixture of 60 g. of amalgamated zinc (mossy or turnings) and 120 ml. of 6 *N* hydrochloric acid is added dropwise over 1 hour a solution of the crude *p*-hydroxybenzylidenimine-C^{13} hydrochloride in 60 ml. of concentrated hydrochloric acid. The mixture is refluxed for 3 hours more, then filtered, diluted with 3–5 volumes of water and extracted with ether. The yield of crude *p*-cresol (Note 8), following evaporation of solvent, is 8.2 g. (75%).

(d) *Silver Acetate-2-C*^{13} (Note 9). To a mixture of 30 g. of chromium trioxide, 25 ml. of water and 150 g. of ice is added a solution of 2 g. of the crude *p*-cresol-α-C^{13} in 25 ml. of concentrated sulfuric acid. The mixture is stored at room temperature for 12 hours, then treated with 75 ml. of concentrated sulfuric acid and refluxed for 3 hours. The acetic-2-C^{13} acid is separated by steam distillation (1 hour), and the distillate is treated with an excess of silver carbonate. The solution is filtered hot and concentrated to crystallize the product; yield 50–70% based on cyanide (Note 10). Degradation studies indicate that the isotope is located only at C-2 (Note 11).

B. Notes

1. Delay results in the mixture turning almost black and in formation of a colored product.

2. The product may be titrated with standard silver nitrate solution. The impurity is mostly sodium chloride, with small amounts of zinc and magnesium chlorides.

3. The procedure is a modification[1] of the procedure of Gattermann[2] for synthesizing hydroxyaromatic aldehydes.

4. The use of potassium chloride to catalyze the Gattermann reaction in the presence of purified zinc cyanide is reported by Arnold.[3]

5. The zinc cyanide dissolves within a few minutes and, after about 20 minutes, a liquid separates which gradually solidifies, when the gas is introduced at a rapid rate.

6. The crude product contains a small amount of *o*-hydroxybenzylidenimine-C^{13} hydrochloride.

7. A review of the Clemmensen reduction is presented by Martin.[4]

8. The crude product contains a small amount of *o*-cresol-α-C^{14}.

9. The procedure is adapted from the method of Kuhn[5] for analysis of organic compounds by oxidation to acetic acid and carbon dioxide.

10. The yield is based on titration of an aliquot of the distillate with standard sodium hydroxide.

11. Directions are given by Anker for converting the silver salt to lithium acetate-2-C^{13} (LiBr), which then is pyrolyzed to 2-propanone-1,3-C$_2^{13}$ (q.v.) having an isotopic content in agreement with that of the acetic-2-C^{13} acid.

[1]R. Adams and I. Levine, J. Am. Chem. Soc., *45*, 2373 (1923).
[2]L. Gattermann, Ann., *357*, 313 (1907).
[3]R. T. Arnold and J. Sprung, J. Am. Chem. Soc., *60*, 1699 (1938).
[4]*Organic Reactions*, Vol. I, Wiley, New York, 1942, P. 155.
[5]R. Kuhn and F. L'Orza, Angew. Chem., *44*, 847 (1931).

SODIUM ACETATE-2-C^{14}

METHOD I

$$C^*H_3I \xrightarrow{Mg} C^*H_3MgI \xrightarrow{CO_2} C^*H_3COOMgI \xrightarrow{H^+}$$

$$C^*H_3COOH \xrightarrow{NaOH} C^*H_3COONa$$

B. M. Tolbert, J. Biol. Chem., 173, 205 (1948).

A. Procedure

The apparatus consists of a high-vacuum manifold fitted with a mercury manometer and vacuum gauge, a storage tube for methyl iodide, sources of dry carbon dioxide and nitrogen, and a three-necked reaction flask. The 150-ml. conical flask is equipped with an overhead induction stirrer and a Dry Ice-acetone reflux condenser (Note 1).

Into the flask containing 0.5 g. of magnesium turnings and 50 ml. of absolute ether, frozen with liquid nitrogen, is vacuum-distilled 2.28 g. (16.1 mmoles) of methyl-C^{14} iodide. The flask is isolated from the system, and the mixture is refluxed for 1 hour (Note 2). The flask is cooled to $-20°$, and pure dry carbon dioxide (Note 3) is admitted from a storage bulb until a pressure of 300 mm. is maintained. The mixture is stirred for 10 minutes: then the flask is opened in a hood, and the complex is decomposed at -20 to $-50°$ with 15 ml. of 6 *N* sulfuric acid. The mixture is treated with 35 ml. of water and 5 g. of silver sulfate (Note 4). The ether is distilled away, and the product is collected by steam-distillation with about 300 ml. of water. The distillate is titrated to pH 8 with *N* sodium hydroxide and a glass electrode, then concentrated to a small volume, filtered and evaporated to dryness. The yield of anhydrous product, dried at 1μ, is 70–75% (Note 5).

METHOD II

$$C^*H_3I \xrightarrow{KCN} C^*H_3CN \xrightarrow{NaOH} C^*H_3COONa$$

J. D. Cox, H. S. Turner and R. J. Warne, J. Chem. Soc., *1950*, 3167.

A. Procedure (Note 6)

Methyl-C^{14} iodide (2.634 g.) is vacuum-distilled into a flask containing 1.7 g. of potassium cyanide (25% excess) and 3 ml. of water. The flask is sealed off at a constriction in the neck (Note 7) and shaken overnight

at room temperature to complete the reaction (Note 8). The mixture is cooled in a Dry Ice-bath, the flask is opened, and the mixture of water, acetonitrile-2-C^{14} and any unchanged methyl-C^{14} iodide is distilled on the vacuum line into a 60-ml. flask containing 2.0 ml. of carbonate-free 20 *N* sodium hydroxide (100% excess). The flask is sealed at another constriction in the neck and heated at 80° for 4 hours. The solution is rinsed into a 100-ml. flask with water; then steam is passed through the hydrolysate for 5 minutes to remove any traces of methanol-C^{14}. The alkaline solution is acidified with sulfuric acid, then treated with 0.4 g. of silver sulfate, and the acetic-2-C^{14} acid is collected by steam distillation. The distillate (1 l.) is titrated with *N* sodium hydroxide (18.44 ml.) to pH 8.8 with use of a glass electrode and pH-meter. The solution is evaporated to dryness, and the product is dried under vacuum at 250°; yield 1.515 g., 99% (Note 9).

Sodium acetate-2-C^{13} is prepared in the same manner from methyl-C^{13} iodide (Note 10).

B. Notes

1. A diagram of the system is presented by Tolbert; also see Figures II,5, II,12 and I, 6. For other types of stirrers and apparatus, see benzoic-C^{14} acid, Note 2.

2. The Grignard reaction is believed to be 100% complete.

3. Purified carbon dioxide is obtained by freezing the gas with liquid nitrogen, evacuating to remove oxygen and nitrogen, then passing the gas through a spiral trap cooled with Dry Ice-acetone to remove moisture.

4. The iodide is precipitated to prevent contamination of the distillate with free iodine.

5. The yield in trial runs was 98–100%. The titrations and weights of dry salt agree within a few tenths per cent.

6. The procedure is an adaptation of Auger's[1] modification of the method of Walden.[2]

7. The long-necked flask is constricted between the connection to the vacuum line and an interjoint for subsequent connection to the line.

8. If the methyl-C^{14} iodide contains ethyl iodide (see methanol-C^{14}), the latter halide is largely unreacted at this stage and the mixture is not homogeneous. The procedure is suitable for scales of 5–50 mmoles, cooling being necessary during larger scale methylations.

9. The product contains less than 0.2% carbonate and no detectable amounts of cyanide, halide, sulfate or formate. A method is indicated for estimating the amount of ethanol contamination in the preparation of the methanol-C^{14}.

10. The isotopic yield is 81% based on carbon dioxide.

C. Other Preparations

By the procedure of Method I is prepared a series of acids; see Table II, 2. Half dozen methods for degrading fatty acids, for assay purposes, are discussed by Dauben;[3] also see Phares,[4] (butyric-4-C^{14} acid, Note 7). Carboxylic acids may be separated and characterized by paper chromatography,[4a] Duclaux distillation,[4b] gas-liquid partition chromatography,[4c] column-chromatography[4d] with an internal indicator,[4e] ion exchange chromatography,[4f] or countercurrent distribution.[4g]

By the procedure of Method II is prepared a series of acids; see Table II, 3.

The preparation of sodium acetate-2-C^{13} by Method II has been reported by Lerner[5] (90%) and by Bloch[6] (66% based on methanol). The latter purified the hydrolysate by heating with permanganate to destroy cyanide and formate. Gianetto[7] has reported a yield of 3.6 g. of sodium acetate-2-C^{14} based on 13 g. of barium carbonate-C^{14}.

The preparation of potassium acetate-2-C^{14}, with an average yield of 87% based on methanol at the 10-mmole scale, has been described by Hess.[8] Methanol-C^{14} was converted through the intermediate methyl-C^{14} hydrogen sulfate (100%) and acetonitrile-2-C^{14} (96%) q.v. The purity was determined by isotope dilution technique to be $100 \pm 1\%$.

A preparation of sodium acetate-2-C^{13} (50–70% yield based on zinc cyanide-C^{13}) has been described by Anker.[9] *p*-Cresol-α-C^{13} (q.v.) was oxidized with hot chromic acid, and the acetic-2-C^{13} acid formed was distilled. The method has been used by Lifson.[10]

[1]M. Auger, Compt. rend., *145*, 1287 (1907).

[2]P. Walden, Ber., *40*, 3124 (1907).

[3]W. B. Dauben, E. Hoerger and J. W. Petersen, J. Am. Chem. Soc., *75*, 2347 (1953).

[4]E. F. Phares, Arch. Biochem. Biophys., *33*, 173 (1951); J. Katz, S. Abraham and I. L. Chaikoff, Anal. Chem., *27*, 155 (1955); B. A. Fry, J. Physiol., *128*, 63P (1955).

[4a]E. P. Kennedy and H. A. Barker, Anal. Chem., *23*, 1033 (1951); A. R. Jones, E. J. Dowling and W. J. Skraba, Anal. Chem., *25*, 394 (1953).

[4b]T. E. Friedmann, J. Biol. Chem., *123*, 161 (1938).

[4c]A. T. James and A. J. P. Martin, Biochem. J., *50*, 679 (1952).

[4d]E. Lederer and M. Lederer, *Chromatography*, Elsevier, New York, 1954, p. 114; S. R. Elsden, Biochem. J., *40*, 252 (1946); E. L. Smith, Biochem. J., *36*, XXII, (1942).

[4e]J. Katz, I. Lieberman and H. A. Barker, J. Biol. Chem., *200*, 431 (1953).

[4f]R. Kunin and R. J. Myers, J. Am. Chem. Soc., *69*, 2874 (1947); H. Busch, R. B. Hurlbert and V. R. Potter, J. Biol. Chem., *196*, 717 (1952); C. W. Davies, Biochem. J., *45*, 38 (1949).

[4g]L. C. Craig, Anal. Chem., *22*, 1346 (1950); *The Technique of Organic Chemistry*, Vol. III, Interscience, New York, 1950, p. 276.

[5]A. B. Lerner, J. Biol. Chem., *181*, 281 (1949).

TABLE II, 2

Synthesis of Acids by the Nitrile Method

Acid	RX	Cyanide	% Yield	Reference	Note
Acetic-1-C^{13}-2-C^{14}	$C^{14}H_3I$	$KC^{13}N$	–	23	–
Propionic-2-C^{13}-3-C^{14}	$C^{14}H_3C^{13}H_2I$	–	–	33, 24	i, j
Propionic-2-C^{14}	$CH_3C^{14}H_2I$	–	–	24a	–
Propionic-3-C^{13}	$C^{13}H_3CH_2I$	–	–	24	i
Hydracrylic-1-C^{11}	$ClCH_2CH_2OH$	$KC^{11}N$	–	25	m
Butyric-1-C^{13}	$CH_3CH_2CH_2I$	$KC^{13}N$	75	26	–
Butyric-2-C^{14}	$CH_3CH_2C^{14}H_2I$	–	85	27	–
3-Butenoic-1-C^{14}	$CH_2{=}CHCH_2Br$	$KC^{14}N$	–	36	o
Valeric-1-C^{13}	$CH_3(CH_2)_3I$	$KC^{13}N$	–	28	i
Valeric-4-C^{14}	$CH_3C^{14}H_2CH_2CH_2I$	–	–	28	i,k
Hexanoic-1-C^{13}	$CH_3(CH_2)_4Br$	$NaC^{13}N$	–	29	–
4-Methylvaleric-2-C^{14}	$(CH_3)_2CH_2CH_2C^{14}H_2Br$	–	55–65	30	l
Octanoic-1-C^{13}	$CH_3(CH_2)_6Br$	$NaC^{13}N$	–	29	–
p-Nitrobenzoic-C^{14}	p-$NO_2C_6H_4Br$	$KC^{14}N$	–	35	n

Notes

i. The purity of the product was checked by determining the partition coefficient[32] and comparing with established values.

j. Conversion of the compound to the lactate, followed by stepwise degradation, showed that there was no isotopic rearrangement.

k. The 1-iodobutane-3-C^{14} was prepared by treating ethylmagnesium-1-C^{14} iodide with ethylene oxide,[33] then decomposing the dry 1-butoxymagnesium-3-C^{13} iodide with hydrogen iodide. Fractional distillation removed ethyl iodide, which arose from scission of the ether bonded to the Grignard complex.

l. The yields by the Grignard synthesis, carbonating at −25°, were only 40%. A mixture of 12.4 mmoles of 1-bromo-3-methylbutane-1-C^{14}, 25 mmoles of potassium cyanide, 0.2 g. of potassium iodide and 25 ml. of ethanol was refluxed for 42 hours. The cooled mixture was treated with 4 g. of silver sulfate and 30 ml. of water; then the 4-methylvaleronitrile-2-C^{14} was distilled. The distillate was refluxed for 24 hours with 15 g. of potassium hydroxide, then acidified with sulfuric acid, and the product was steam-distilled. The molar specific activity was 96.3% of the calculated value based on sodium 3-methylbutyrate-1-C^{14}, from which the halide was prepared in 73% yield, thus demonstrating the absence of contamination in the preparation of carbinols by high-pressure hydrogenolysis.[30]

m. The compound has been used in studying the complex oxidative degradation[25,34] of propionic acid by alkaline permanganate, which yields one mole each of carbonate and oxalate. About 70% of the carbonate originates from C-3.

n. The condensation is accomplished in dry pyridine, giving the intermediate *p*-nitrobenzonitrile-C^{14}. The acid was used in a study of the mechanism of anionotropic rearrangement,[35] 1-phenyl-2-propenyl *p*-nitrobenzoate-C^{14} forming both cinnamyl *p*-nitrobenzoate-C^{14} and 1-phenyl-2-propenyl *p*-nitrobenzoate-C^{14} by allylic rearrangement and by exchange, respectively, in aqueous or nonaqueous solvents.

o. The 3-butenonitrile-1-C^{14}, formed by condensation in a sealed tube at 100°, is hydrolyzed, and isomerized to crotonic-1-C^{14} acid by boiling for 5 minutes with 50% sulfuric acid; over-all yield 25% (the isomerization yield[37] is 98% on the usual laboratory scale). Swan[38] has reported the preparation of crotonic-1-C^{14} acid from malonic-1-C^{14} acid and acetaldehyde by the method of Letch[39] (the usual yield[39] at the laboratory scale is 86%, m.p. 72°). Also see benzoic-C^{14} acid, Table II, 1.

[6]I. Zabin and K. Bloch, *ibid.*, *185*, 117 (1950); H. N. Little and K. Bloch, *ibid.*, *183*, 33 (1950).

[7]R. Gianetto and L. P. Bouthillier, Can. J. Biochem. and Physiol., *32*, 154 (1954).

[8]D. N. Hess, J. Am. Chem. Soc., *73*, 4038 (1951); F. Kögl, P. Emmelot and D. H. W. den Boer, Ann., *589*, 1 (1954).

[9]H. S. Anker, J. Biol. Chem., *166*, 219 (1946).

[10]N. Lifson, V. Lorber, W. Sakami and H. G. Wood, J. Biol. Chem. *176*, 1263 (1948).

[11]M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 193.

[12]V. Lorber, N. Lifson, H. G. Wood, W. Sakami and W. W. Shreeve, J. Biol. Chem., *183*, 517 (1950).

[13]M. Kleiber, A. L. Black, M. A. Brown and B. M. Tolbert, J. Biol. Chem., *203*, 339 (1953).

[14]A. Finkelstein and W. A. Noyes, Jr., Discussions Faraday Soc., *14*, 81 (1953).

[15]R. Ostwald, P. T. Adams and B. M. Tolbert, J. Am. Chem. Soc., *74*, 2425 (1952).

[16]D. M. Hughes, R. Ostwald and B. M. Tolbert, J. Am. Chem. Soc., *74*, 2434 (1952).

[17]P. Adams and B. M. Tolbert, Nucleonics, 7 (3), 60 (1950).

[18]N. Lifson, V. Lorber, W. Sakami and H. G. Wood, J. Biol. Chem., *176*, 1263 (1948).

[19]M. J. Coon and S. Gurin, J. Biol. Chem., *180*, 1159 (1949).

[20]I. Gray, P. Adams and H. Hauptmann, Experientia, *6*, 430 (1950).

[21]R. P. Geyer, M. Cunningham and J. Pendergast, J. Biol. Chem., *185*, 461 (1950); *Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 25.

[22]G. W. Brown, Jr., D. D. Chapman, H. R. Matheson, I. L. Chaikoff and W. G. Dauben, J. Biol. Chem., *209*, 537 (1954).

[23]V. Lorber, N. Lifson, H. G. Wood, W. Sakami and W. W. Shreeve, J. Biol. Chem., *183*, 517 (1950).

[24]V. Lorber, N. Lifson, W. Sakami and H. G. Wood, J. Biol. Chem., *183*, 531 (1950).

[24a]R. M. Roberts and S. G. Brandenberger, Chemistry and Ind.(London), *1955*, 227.

[25]P. Nahinsky, C. N. Rice, S. Ruben and M. D. Kamen, J. Am. Chem. Soc., *64*, 2299 (1942).

[26]I. Zabin and K. Bloch, J. Biol. Chem., *192*, 261 (1951).

[27]P. Adams and B. M. Tolbert, Nucleonics, 7 (3), 60 (1950); M. Kleiber, A. L. Blach, M. A. Brown, J. Luick, C. F. Baxter and B. M. Tolbert, J. Biol. Chem., *210*, 239 (1954).

[28]I. Siegel and V. Lorber, J. Biol. Chem., *189*, 571 (1951).

[29]D. I. Crandall and S. Gurin, J. Biol. Chem., *181*, 829 (1949).

[30]H. Hauptmann, P. T. Adams and B. M. Tolbert, J. Am. Chem. Soc. *74*, 2423 (1952).

[31]B. M. Tolbert, F. Christenson, F. N. Chang and P. P. T. Sah, J. Org. Chem., *14*, 525 (1949).

[32]O. L. Osborn, H. G. Wood and C. H. Werkman, Ind. Eng. Chem., Anal. Ed., *8*, 270 (1936); *ibid.*, *5*, 247 (1933).

[33]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 306.

[34]H. G. Wood, C. H. Werkman, A. Hemingway, A. O. Nier and C. G. Stuckwisch, J. Am. Chem. Soc., *63*, 2140 (1941); P. Nahinsky and S. Ruben, *ibid.*, *63*, 2275 (1941).

[35]E. A. Braude, D. W. Turner and E. S. Waight, Nature, *173*, 863 (1954).

[36]O. Simamura, N. Inamoto and T. Suehiro, Bull. Chem. Soc. Japan, *27*, 221 (1954); through Chem. Abstracts, *49*, 7494 (1955).
[37]E. J. Boorman and R. P. Linstead, J. Chem. Soc., *1933*, 577.
[38]G. A. Swan, J. Chem. Soc., *1955*, 1039.
[39]R. A. Letch and R. P. Linstead, J. Chem. Soc., *1932*, 454.

SODIUM ACETATE-C_2^{14}

$$BaC^*O_3 \xrightarrow{Mg} BaC^*_2 \xrightarrow{H_2O} HC^*{\equiv}C^*H \xrightarrow[2.\ H^+]{1.\ KOH} \underset{3.\ NaOH}{} C^*H_3C^*OONa$$

R. Abrams, Experientia, 3, 488 (1947).

A. Procedure (Note 1)

An intimate mixture of 0.2 g. (1 mmole) of barium carbonate-C^{14} and 0.5 g. of magnesium powder is compressed to a small wafer in a steel pellet press (Note 2). The pellet is heated in a stream of hydrogen in an iron boat within an iron pipe placed in a furnace at 1000°. When reaction occurs and the pellet explodes (Note 3), the pipe is cooled rapidly with water and the gray-black contents of the boat are transferred into an acetylene generator. The apparatus (Figure II, 2) consists of a flask fitted with a dropping funnel and connected through a side arm to an evacuated, liquid nitrogen-cooled tube containing 0.3 g. of asbestos upon which has been dried 0.6 g. of potassium hydroxide. The evacuated system is isolated from the pump, 15 ml. of water is added to the barium carbide-C_2^{14}, and the mixture is warmed with a flame for 5–10 minutes to ensure complete evolution of acetylene-C_2^{14}, which is condensed with some water in the cold receiver (Note 4). The tube is sealed off at a constriction in the neck and heated for 1 hour in a furnace at 250°. The reaction mixture is transferred into a distilling flask with 50 ml. of water, then acidified with 6 ml. of 20% sulfuric acid, and the product is steam-distilled. The distillate is titrated with standard sodium hydroxide solution; yield 60–80% based on barium carbonate (Note 5).

B. Notes

1. The procedure is an adaptation of the method of Strosacker.[1] A one-piece glass apparatus for the isotopic preparation is described by Ruyssen and Loos;[2] yield 60–80% based on 0.02 g. of barium carbonate.

2. The reduction of barium carbonate by the method of Cramer and Kistiakowsky (see acetylene-C_2^{14}, Other Preparations) goes well at the 1-mmole scale, only if advantage is taken of the heat of reaction by forming the reactants into a hard, dense mass.

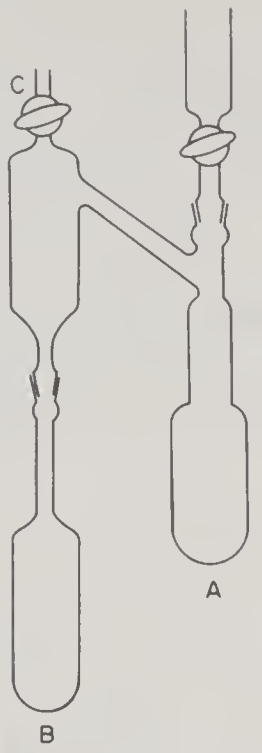


Fig. II, 2. Apparatus for the conversion of barium carbide to acetate (R. Abrams). A, acetylene generator; B, potassium hydroxide-on-asbestos; C, outlet to vacuum.

3. Reaction occurs when the pipe reaches 700–750°.

4. Acetylene titrations of trial preparations, according to the method of Willstätter,[3] indicate yields are 75–85%.

5. The conversion of acetylene is 80–90%. The acid was identified by a Duclaux titration.

C. Other Preparations

Acetic-C_2^{14} and acetic-C_2^{13} acids have been prepared by Gurin[4] through oxidation of the aldehyde with excess silver oxide; yield 40% based on barium carbonate.

Acetic-C_2^{13} acid has been prepared by Lifson[5,6] and Wood,[7] by oxidizing acetaldehyde-C_2^{13} with dichromate at the 50-mmole level. The product purity was checked by determination of neutralization equivalent, total carbon and partition coefficient.[8]

[1]C. J. Strosacker, C. C. Kennedy and E. L. Pelton, U. S. 1,866,430; Chem. Abstracts, *26*, 4345 (1932).

[2]R. Ruyssen and R. Loos, Mededeel. Koninkl. Vlaam. Acad. Wetenschap., Belg., *11* (6), 5 (1949); Chem. Abstracts, *44*, 1900 (1950).

[3]R. Willstätter and E. Maschmann, Ber., *53*, 939 (1920).

[4]S. Gurin and A. M. Delluva, J. Biol. Chem., *170*, 545 (1947); W. Sakami, W. E. Evans and S. Gurin, J. Am. Chem. Soc., *69*, 1110 (1947).

[5]N. Lifson, V. Lorber, W. Sakami and H. G. Wood, J. Biol. Chem., *176*, 1263 (1948).

[6]J. S. Lee and N. Lifson, J. Biol. Chem., *193*, 253 (1951).

[7]H. G. Wood, J. Biol. Chem., *194*, 905 (1952); G. L. Stahly, O. L. Osborn and C. H. Werkman, Analyst, *59*, 319 (1934).

[8]O. L. Osborn, H. G. Wood and G. H. Werkman, Ind. Eng. Chem., Anal. Ed., *5*, 247 (1933).

PROPIONIC-2-C^{14} ACID

METHOD I

$$CH_3C^*H_2MgX \xrightarrow{CO_2} CH_3C^*H_2COOH$$

See sodium acetate-2-C^{14}, Method I, Table II.

METHOD II

$$CH_3C^*OCOOH \xrightarrow{NH_2NH_2} CH_3\overset{\overset{NNH_2}{\|}}{C}{}^*COOH \xrightarrow{KOH} CH_3C^*H_2COOH$$

E. H. Mosbach, E. F. Phares and S. F. Carson, J. Am. Chem. Soc., *73*, 5477 (1951).

A. Procedure (Note 1).

A mixture of 0.85 mmole of pyruvic-2-C^{14} acid, 5 ml. of water, 25 ml. of redistilled diethylene glycol, 5 ml. of 85% hydrazine solution and 1 g. of potassium hydroxide is refluxed for 1 hour in a 200-ml. flask. Water is distilled off until the temperature reaches 190°; then refluxing is continued for another hour. The cooled mixture is acidified with sulfuric acid and steam-distilled. The distillate is concentrated, and the product is purified (Note 2) by partition chromatography (Note 3). The yield of sodium salt is 73%. Degradation studies show that there is no rearrangement (Note 4).

B. Notes

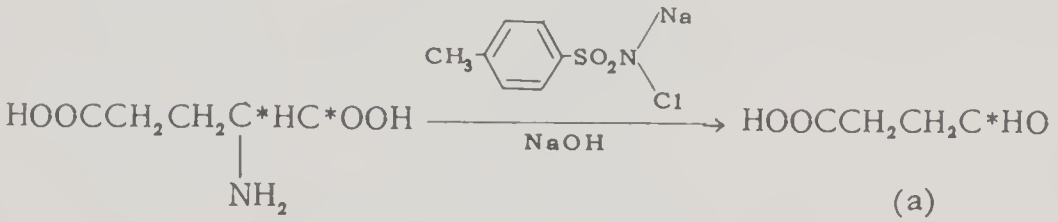
1. The procedure is the Huang-Minlon modification of the Wolff-Kishner reduction of aldehydo and keto acids; see butyric-4-C^{14} acid.

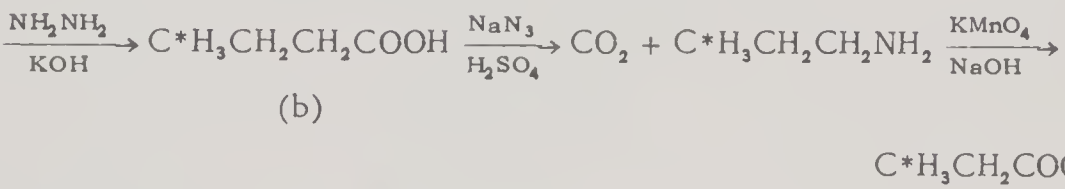
2. Traces of acetic acid are formed by thermal decomposition of the solvent. The product is characterized by its position on the chromatogram, Duclaux distillation and preparation of the *p*-bromophenacyl ester, m.p. 59-60°.

3. The chromatographic procedure is described in detail by Peterson and Johnson; see butyric-4-C^{14} acid.

4. A stepwise degradation is described by Phares; see butyric-4-C^{14} acid, Note 7.

BUTYRIC-4-C^{14} ACID





E. H. Mosbach, E. F. Phares and S. F. Carson, J. Am. Chem. Soc., *73*, 5477 (1951); Arch. Biochem. Biophys., *33*, 179 (1951).

A. Procedure

(a) *Succinaldehydic-4-C^{14} Acid, (3-Formyl-C^{14}-propionic Acid)* (Note 1). Glutamic-1,2-C_2^{14} acid is oxidized with an equimolecular amount of chloramine-T, according to the procedure of Dakin,[1] which follows. The product is used directly in solution; the yield is quantitative (Note 2).

To a stirred solution of 1.47 g. of glutamic acid in 100 ml. of 0.1 *N* sodium hydroxide, through which nitrogen is bubbled, is added 2.9 g. of chloramine-T, causing *p*-toluenesulfonamide to precipitate. A steady evolution of ammonia and carbon dioxide is facilitated by warming to 50°, active chlorine rapidly disappears in about 20 minutes (Note 3). The mixture is cooled, and the sulfonamide is removed by filtration and washed. The filtrate is treated with a solution of 1.7 g. of *p*-nitrophenylhydrazine in hot dilute hydrochloric acid. The yield of the *p*-nitrophenylhydrazone is 2.1 g. (88.5%); purification is effected by recrystallization from water.

(b) *Butyric-4-C^{14} Acid.* An aqueous solution of 0.85 mmole of the sodium salt of the aldehydic acid, concentrated to 5 ml. in a 200-ml. flask, is treated with 25 ml. of redistilled diethylene glycol (Note 4), 5 ml. of 85% hydrazine solution and 1 g. of potassium hydroxide. The solution is refluxed for 1 hour, water is distilled off until the vapor temperature reaches 190°, and then refluxing is continued for 1 hour. The cooled mixture is acidified with 10 *N* sulfuric acid and steam-distilled until all volatile acid is collected. The distillate is concentrated, and the product is isolated by partition chromatography (Note 5) on a column of Celite moistened with 0.5 *N* sulfuric acid (nonmobile phase)

and developed with 1% 1-butanol in chloroform (mobile phase) saturated with the nonmobile phase; yield 45–55% based on glutamic acid (Note 6). Degradation studies (Note 7) indicate that no carbon rearrangement occurs under the conditions of the Huang-Minlon modification of the Wolff-Kishner reduction.[2]

B. Notes

1. Owing to its instability, succinaldehydic acid is prepared as needed.

2. The succinaldehydic-4-C^{14} acid is characterized and assayed by conversion to the *p*-nitrophenylhydrazone, m.p. 178–180°, and the 2,4-dinitrophenylhydrazone, m.p. 198–200°. The aldehyde may be isolated and distilled only with considerable loss. In the presence of two molecules of chloramine-T, the product is 3-cyanopropionic acid.

3. The presence of chlorine is indicated by starch, potassium iodide and acetic acid.

4. Triethylene glycol cannot be used, as it yields small amounts of butyric and other acids. Traces of acetic acid are formed by thermal decomposition of diethylene glycol under the conditions of the reduction, thus necessitating the chromatographic step.

5. A procedure for the chromatographic separation of carboxylic acids, described by Marvel and Rands,[3] employs water adsorbed on silicic acid as the immobile phase and solutions of butanol-chloroform as the moving phase. The eluant is made progressively more polar with increasing concentrations of butanol in order to develop the more water-soluble acids.

A procedure for separating fatty acids on a column of Celite is described in detail by Peterson.[4] With a benzene–water system, a mixture of formic, acetic and propionic acids may be separated quantitatively and estimated in the presence of any number of saturated fatty acids having 4 or more carbon atoms. The recovery of butyric acid from a mixture is 98.2–98.6%. The acids are eluted in the order of decreasing molecular weight. A nonmobile phase of 27–35 *N* sulfuric acid, which is a better solvent than water for fatty acids of intermediate chain length, permits a separation of those acids. Another separation is described by Bueding.[5]

6. The product is characterized by its position on the chromatogram, Duclaux distillation and preparation of the *p*-bromophenacyl ester, m.p. 63–64.5°.

7. The general stepwise degradation involves decarboxylation by the Schmidt reaction (93–95% yield), followed by permanganate oxidation of the resulting amine to an acid of one less carbon (50–60% yield).[6, 7]

The apparatus is illustrated in Figures II, 3 and II, 4. To a solution of 0.5 mmole of sodium salt (vacuum-dried) and 0.3 ml. of 100% sul-

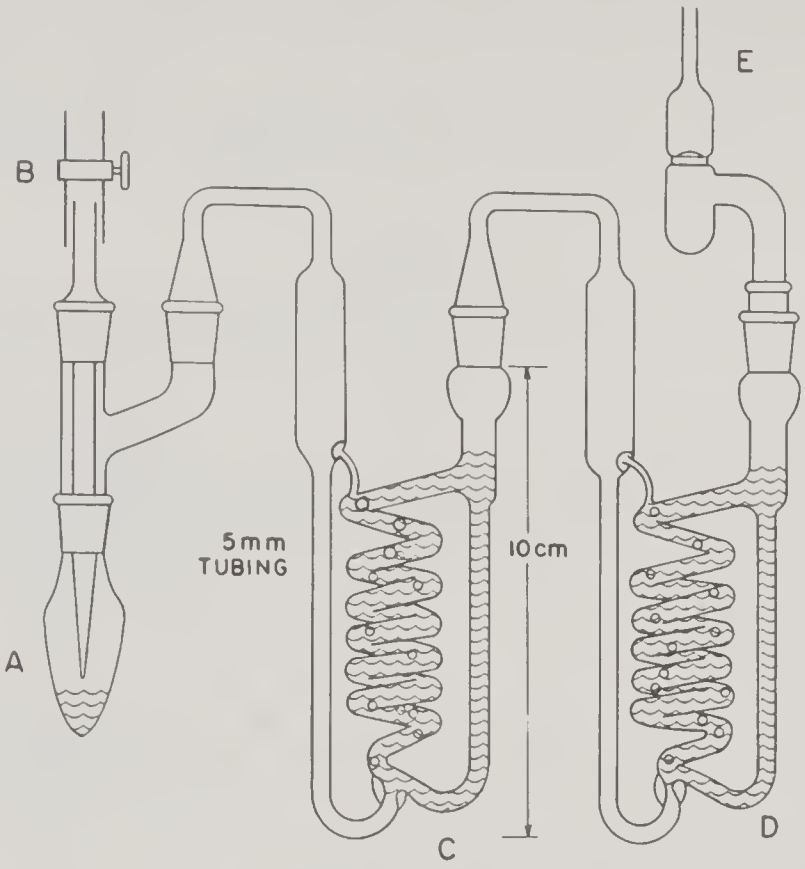


Fig. II, 3. Acid decarboxylation apparatus (E. F. Phares). A, reaction flask; B, inlet for carbon dioxide-free air; C, bubbler containing 5% potassium permanganate in 1 *N* sulfuric acid; D, bubbler containing 0.5 *N* sodium hydroxide; E, outlet to vacuum through a mercury check valve.

furic acid[6] at 15° is added 0.050 g. (0.77 mmole) of sodium azide, and the mixture is dissolved by shaking. The flask is attached to the traps and heated at 35–70° during 30 minutes. The system is swept with air for 10 minutes, and the trapped carbon dioxide is precipitated as barium carbonate for assay. The permanganate solution in the first scrubber is replaced with 5.0 ml. of 0.2 *N* sulfuric acid, 5 *N* sodium hydroxide is added through the sweep-tube, and the amine is collected by sweeping the reaction tube for 15 minutes at 90–100° with carbon dioxide-free air.

A mixture of 0.2 mmole of amine sulfate, 3.0 ml. of 5% potassium permanganate and 2 mmoles of excess sodium hydroxide is heated at 90–100° for 15 minutes (or 1 hour at room temperature) in a low-pressure reaction flask. The solution is acidified with sulfuric acid and steam-distilled, the distillate being titrated and evaporated. In the case of methylamine, sulfuric acid is added through the sweep-tube, and the system is swept for 15 minutes. The small amounts of lower fatty acids, which contaminate the product acid in the oxidation of amines higher than ethylamine, may be separated by partition chromatography.

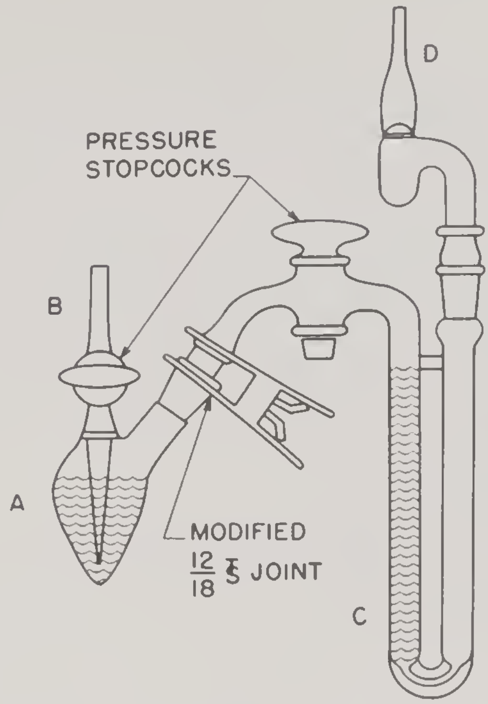


Fig. II, 4. Amine oxidation apparatus (E. F. Phares). A, reaction flask; B, inlet for carbon dioxide-free air; C, trap containing 0.5 *N* sodium hydroxide; D, outlet to vacuum through mercury check valve.

Methylamine-C^{14}, ethylamine-1[and 2]-C^{14} and propylamine-3-C^{14} have been obtained[6] by degradation of the appropriate acids.

[1] H. D. Dakin, Biochem. J., *11*, 79 (1917).
[2] *Organic Reactions,* Vol. IV, Wiley, New York, 1946, Chapter 8, p. 385.
[3] C. S. Marvel and R. D. Rands, Jr., J. Am. Chem. Soc., *72*, 2642 (1950).
[4] M. H. Peterson and M. J. Johnson, J. Biol. Chem., *174*, 775 (1948).
[5] E. Bueding and H. W. Yale, J. Biol. Chem., *193*, 411 (1951).
[6] E. F. Phares, Arch. Biochem. Biophys., *33*, 173 (1951); E. H. Mosbach, E. F. Phares and S. F. Carson, *ibid.*, *33*, 179 (1951).
[7] B. A. Fry, J. Physiol., *128*, 63P (1955); C. Scheurch and E. H. Huntress, J. Am. Chem. Soc., *71*, 2233 (1949).

2-METHYL-C¹⁴-BUTYRIC ACID

$$C^*H_3I \xrightarrow[NaOC_2H_5]{CH_3CH_2CH(COOC_2H_5)_2} CH_3CH_2\underset{\displaystyle C^*H_3}{\underset{|}{C}}(COOC_2H_5)_2 \xrightarrow[(HCl)]{KOH}$$

$$CH_3CH_2\underset{\displaystyle C^*H_3}{\underset{|}{C}}(COOH)_2 \xrightarrow[\Delta]{} CH_3CH_2\underset{\displaystyle C^*H_3}{\underset{|}{C}}HCOOH$$

M. J. Coon, N. S. B. Abrahamsen and G. S. Greene, J. Biol. Chem., 199, 75 (1952).

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A. Procedure (Note 1)

To a solution of 58 mmoles of sodium ethoxide in absolute ethanol is added 58 mmoles of ethyl ethylmalonate. To the chilled, vigorously stirred mixture is added slowly 55 mmoles of methyl-C^{14} iodide. The solution is refluxed for 30 minutes, then treated with an excess of non-isotopic methyl iodide (106 mmoles), and the heating is continued for 30 minutes. The solvent is distilled, and the residual ethyl ethylmethyl-C^{14}-malonate is refluxed for 6 hours with 20 g. of potassium hydroxide in 20 ml. of water. The mixture is chilled, acidified and extracted with ether. The extract of ethylmethyl-C^{14}-malonic acid is shaken with clean mercury and evaporated to dryness.

The dry acid is decarboxylated by heating in a flask at 190°, and the product is collected by vacuum distillation (Note 2); yield 57% based on the ethylmalonic ester (Note 3).

B. Notes

1. The procedure is adapted from the malonic ester condensation described by Conrad and Bischoff;[1] also see Adams and Kamm,[2] Dox,[3] and Reid and Ruhoff.[4]

2. The acid (b.p. 175°) is characterized by a determination of the Duclaux constants and by preparation of the *p*-bromophenacyl ester, m. p. 53° (cor.).

3. The yield is 80% at the 200-mmole scale.

C. Other Preparations

A number of acids have been prepared by the malonic ester synthesis. Butyric-3-C^{14} acid has been prepared from ethyl-1-C^{14} iodide by Coon[5] (yield 41%), and Zabin[6] (yield 51% based on ethanol-1-C^{14}). The latter investigators distilled the product azeotropically with benzene and toluene, according to the method developed by Schicktanz[7] for the simultaneous qualitative and approximate quantitative determination of simple aliphatic acids in mixture. Benzene removes the acetic acid arising from ethyl malonate, and the product is distilled with toluene. Sodium butyrate-3-C^{14} has also been separated[8] from acetate by chromatography on a column of neutral Celite and elution with 1% 1-butanol in chloroform.

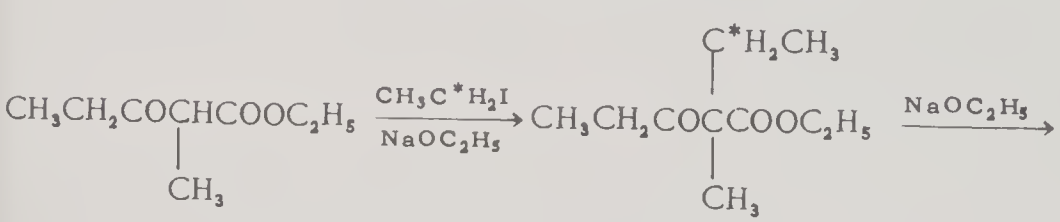
Butyric-3-C^{13} acid has been similarly prepared[9] and purified by heating the potassium salt with methyl sulfate, then fractionating and saponifying the methyl ester; yield 24.5 mmoles (25% based on 93.5 mmoles of sodium acetate-1-C^{13}), mixed with 0.96 mmole of acetic acid arising from the malonic ester.

Octanoic-3-C^{13} acid has been prepared by Crandall[3,10] from 1-bromohexane-1-C^{13} through the intermediate ethyl hexyl-1-C^{13}-malonate. Preparation of the 3-C^{14} compounds has been reported by Geyer.[11]

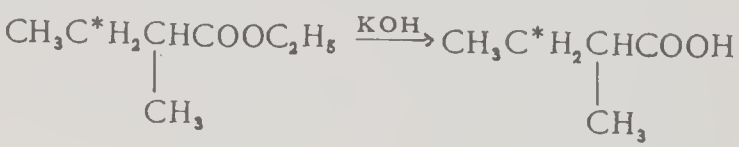
Hexadecanoic-3-C^{14} acid has been prepared[12] from 1-bromotetradecane-1-C^{14}.

[1]M. Conrad and C. A. Bischoff, Ann., *204*, 143 (1880).
[2]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 250.
[3]A. W. Dox, J. Am. Chem. Soc., *46*, 1707 (1924).
[4]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 474.
[5]M. J. Coon and N. S. B. Abrahamsen, J. Biol. Chem., *195*, 805 (1952).
[6]I. Zabin and K. Bloch, J. Biol. Chem., *192*, 261 (1951); C. A. Bischoff, Ber., *28*, 2622 (1895).
[7]S. T. Schicktanz, W. I. Steeles and A. C. Blaisdell, Ind. Eng. Chem., Anal. Ed., *12*, 320 (1940).
[8]V. Lorber and M. Cook, J. Biol. Chem., *215*, 823 (1955).
[9]N. Lifson, V. Lorber, W. Sakami and H. G. Wood, J. Biol. Chem., *176*, 1263 (1948).
[10]D. I. Crandall and S. Gurin, J. Biol. Chem., *181*, 829 (1949).
[11]R. P. Geyer, M. Cunningham and J. Pendergast, J. Biol. Chem., *185*, 461 (1950).
[12]G. W. Brown, Jr., D. D. Chapman, H. R. Matheson, I. L. Chaikoff and W. G. Dauben, J. Biol. Chem., *209*, 537 (1954).

2-METHYLBUTYRIC-3-C^{14} ACID



(a)



(b) (c)

M. J. Coon and N. S. B. Abrahamsen, J. Biol. Chem., *195*, 805 (1952).

A. Procedure (Note 1)

(a) *Ethyl 2-Ethyl-1-C^{14}-2-methyl-3-oxovalerate* (Note 1). To a stirred mixture of 9.2 g. of ethyl 2-methyl-3-oxovalerate (Note 2) and 9.1 g. of ethyl-1-C^{14} iodide is added dropwise over 30 minutes a solution of 1.34 g. of sodium in 25 ml. of absolute alcohol. The mixture is refluxed on a steam-bath for 6 hours.

(b) *Ethyl 2-Methylbutyrate-3-C^{14}* (Note 3). The alcoholic solution of ethyl 2-ethyl-1-C^{14}-2-methyl-3-oxovalerate is heated for 1.5 hours with sufficient alcoholic sodium ethoxide to make it alkaline (Note 4).

(c) *2-Methylbutyric-3-C^{14} Acid.* The ethyl 2-methylbutyrate-3-C^{14} reaction mixture is refluxed for 2.5 hours with 40 ml. of a 15% solution of potassium hydroxide in 80% alcohol. The solvents are removed by repeated distillation with added benzene; then the residue is cooled, acidified and extracted with ether. The dried extract is fractionally distilled; yield 78% based on ethyl iodide (Note 5).

B. Notes

1. The method is a modification of the methods of Israel[1] and Dieckmann.[2] Ethyl 2-ethyl-2-methyl-3-oxovalerate boils at 97–98° (16 mm.).

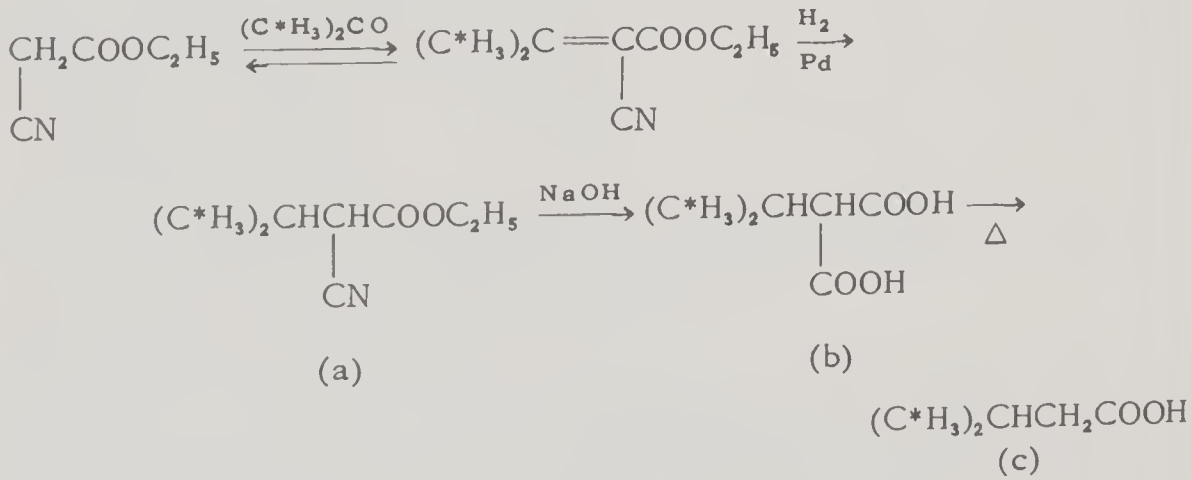
2. Directions are given by McElvain[3] for preparing ethyl 2-methyl-3-oxovalerate by the acetoacetic ester condensation.

3. The procedure is adapted from the catalytic method of cleaving mono- and dialkylacetoacetic esters into the corresponding mono- and dialkylacetic esters described by Dieckmann;[2] also see Saur[4] and Israel.[1]

4. The ester[4] boils at 132–133°.

[1]A. Israel, Ann., *231*, 197 (1885).
[2]W. Dieckmann, Ber., *33*, 2670 (1900).
[3]S. M. McElvain, J. Am. Chem. Soc., *51*, 3124 (1929); *Organic Reactions*, Vol. I, Wiley, 1942, Chapter 9.
[4]R. Saur, Ann., *188*, 257 (1877).

3-METHYL-C^{13}-BUTYRIC-4-C^{13} ACID



I. Zabin and K. Bloch, J. Biol. Chem., *185*, 117 (1950).

A. Procedure

(a) *Ethyl 2-Cyano-3-methyl-C^{13}-butyrate-4-C^{13}* (Note 1). To a mixture of 0.12 g. of palladium-black, 0.23 g. of ammonium acetate and 0.34 ml. of glacial acetic acid, in a small three-necked flask equipped with a dropping funnel, hydrogen inlet and stopper, is added 2-propanone-1,3-C_2^{14} (Note 2) and 15 ml. of absolute alcohol. The flask is flushed with hydrogen; then 1 ml. of ethyl cyanoacetate is added through the dropping funnel, and the mixture is shaken for hydrogenation at 1–2 atmospheres (Note 3). The remainder of the ethyl cyanoacetate is added in 1-ml. portions at 2-hour intervals (Note 4). When the hydrogen uptake ceases (9.5 hours), the catalyst is filtered off and washed.

(b) *(1-Methyl-C^{13}-ethyl-2-C^{13})malonic Acid.* The solution of ethyl 2-cyano-3-methyl-C^{13}-butyrate-4-C^{13} is refluxed for 26 hours with 9 g. of sodium hydroxide in 25 ml. of water. The alcohol is distilled off, the solution is acidified with sulfuric acid, and the product is collected by continuous ether extraction for 9 hours. The extract is dried over sodium sulfate, and the solvent is distilled.

(c) *3-Methyl-C^{13}-butyric-4-C^{13} Acid.* The crude isopropylmalonic acid is decarboxylated by heating at atmospheric pressure in a fractionating still, and the product is collected, b.p. 175–180°; yield 65–76% based on acetone (Note 5). The isotope concentration agrees with the calculated value based on methyl-C^{13} iodide.

B. Notes

1. The procedure is an adaptation of the method of Alexander and Cope[1] for preparing ethyl monoalkylcyanoacetates.

2. The 2-propanone-1,3-C_2^{14} is presumed to be that prepared from 2.5 g. (or a multiple thereof) of lithium acetate-2-C^{13}, in 80–95% yield.

3. The reduction is mildly exothermic.

4. The ethyl cyanoacetate is in excess over the ketone.

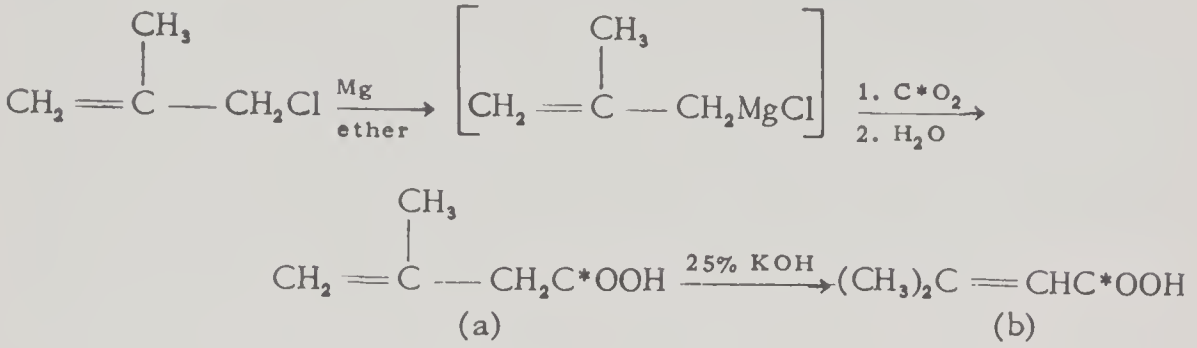
5. The neutral equivalent is 103, calc'd. 102.

C. Other Preparations

By a similar procedure, starting with 2-propanone-2-C^{14}, are prepared:[2] ethyl 2-cyano-3-methylbutyrate-3-C^{14}, b.p. 89–91° (8 mm.);[1] (1-methyl-ethyl-1-C^{14})malonic acid, and 3-methylbutyric-3-C^{14} acid (yield 51% based on acetone).

[1]E. R. Alexander and A. C. Cope, J. Am. Chem. Soc., 66, 886 (1944).
[2]M. J. Coon, J. Biol. Chem., 187, 71 (1950).

3-METHYLCROTONIC-1-C^{14} ACID
(Senecioic-C^{14} Acid)



D. L. Williams and D. G. Ott, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, unpublished work.

A. Procedure

(a) *3-Methyl-3-butenoic-1-C^{14} Acid.* A solution of 4.4 mmoles of methallylmagnesium chloride in 31.2 ml. of ether is transferred, under nitrogen, into the reaction flask of a vacuum-type carbonation apparatus. The solution is frozen in a liquid nitrogen-bath, and the apparatus, including a carbon dioxide generator, is evacuated. The solution is thawed, refrozen and again placed under vacuum. Then the carbon-C^{14} dioxide is generated from 1.1 mmoles of barium carbonate-C^{14} and transferred into the flask with the frozen Grignard solution. The latter is allowed to melt in a bath at -80°. This bath is then replaced with a bath at -60° (Note 1), and the solution is stirred magnetically for 15 minutes. With continued stirring, 5 ml. of water is added dropwise, the solution is rapidly warmed with a water-bath, and 5 ml. of 50% potassium hydroxide solution is added. The resulting mixture, containing the 3-methyl-3-butenoic-1-C^{14} acid, is transferred to a 100-ml. 2-necked flask by washing of the reaction flask with a total of 9 ml. of 25% potassium hydroxide solution and 8 ml. of water.

(b) *Senecioic-C^{14} Acid, (3-Methyl-2-butenoic-1-C^{14} Acid).* The ether is distilled off with an oil-bath which is then raised to a temperature of $130-140^\circ$ as 8 ml. of water and a small amount of oily by-product are also removed by distillation (Note 2). The condenser is then adjusted for reflux, and the mixture is heated for an additional 2.5 hours (Note 3). The mixture is cooled in an ice-bath, and 10 ml. of 80% sulfuric acid and 1.37 g. of silver sulfate are added with stirring (Note 4). The product is then isolated by steam distillation; a total of 240 ml. of distillate is collected. Titration of the distillate with standard sodium hydroxide solution indicates the yield of organic acid to be 93.8% (Note 5). The

solution is evaporated to dryness with a stream of nitrogen and an infrared lamp. The residue of sodium salts is then cooled in a bath at 0° and treated with 1.5 ml. of 1 *N* sulfuric acid. The mixture is cooled at 0° for 30 minutes to effect complete crystallization of the senecioic-C^{14} acid. The mother liquor is removed with a filter stick, and the crystalline product is washed with 1 ml. of 1 *N* sulfuric acid (Note 6). The product is again steam-distilled, and titration of the distillate indicates the yield to be 53.5% based on barium carbonate-C^{14} (Note 7).

B. Notes

1. Preliminary experiments indicates that −60° was the optimum temperature for a high yield of acid.

2. Thus the final concentration of potassium hydroxide is 25%.

3. According to Goldberg and Linstead,[1] vinylacetic acid ($\beta\gamma$ unsaturated) isomerizes irreversibly in 25% potassium hydroxide solution at 100° to form crotonic acid ($\alpha\beta$ unsaturated). Morton, *et al.*,[2] prepared 3-methyl-3-butenoic acid, m.p. ~ 20°, b.p. 49° (3 mm.), n_D^{20} 1.4328, by metalation of isobutene with amylsodium and carbonation of the product. The 3-methyl-3-butenoic acid was isomerized to 3-methyl-2-butenoic acid (senecioic acid), m.p. 68.9–69.3°, by treatment with 25% potassium hydroxide at 100°.

4. Silver sulfate was added to prevent distillation of hydrobromic acid with the product.

5. A sample of this solution was chromatographed on Whatman No. 1 paper strips with butanol saturated with water. A radioautograph of the chromatogram indicated the product to be a mixture of 3-methyl-3-butenoic-1-C^{14} acid and the desired senecioic-C^{14} acid. In preliminary experiments, 3-methyl-3-butenoic acid, m.p. ~ 20°, was isolated by ether extraction and then isomerized to senecioic acid, m.p. 66–69°.

6. Since the senecioic acid is quite volatile and the preparation was to be used as an aqueous solution of the sodium salt, the crystalline senecioic-C^{14} acid was not dried. In preliminary experiments, 1.1 mmoles of senecioic acid was recrystallized in this manner and steam-distilled. Titration of the distillate indicated a recovery of 83%.

7. A paper chromatogram of an aliquot of this solution showed the presence of only 1 compound. The mother liquor from the recrystallization was also steam-distilled; the distillate contained 0.38 mmole (34.5%) of acid. A paper chromatogram of this solution indicated the presence of both the intermediate acid and the final product.

[1] A. G. Goldberg and R. P. Linstead, J. Chem. Soc., *1928*, 2343.

[2] A. A. Morton, M. L. Brown, M. E. T. Holden, R. L. Letsinger and E. E. Magat, J. Am. Chem. Soc., 67, 2224 (1945).

3-METHYLCROTONIC-3-C^{14} ACID
(C_1^{14}-Senecioic Acid)

$$BrZnCH_2COOC_2H_5 \xrightarrow{(CH_3)_2C{*}O} (CH_3)_2C^{*}(OH)CH_2COOC_2H_5 \xrightarrow[\Delta]{KHSO_4}$$

(a)

$$(CH_3)_2C^{*}{=}CHCOOC_2H_5 \xrightarrow{NaOH} (CH_3)_2C^{*}{=}CHCOOH$$

(b)

K. Bloch, L. C. Clark and I. Harary, J. Biol. Chem., *211*, 687 (1954).

A. Procedure

(a) *Ethyl 3-Hydroxy-3-methylbutyrate-3-C^{14}* (Note 1). 2-Propanone-2-C^{14} (20 mmoles) is reacted with 22 mmoles of ethyl bromoacetate in 20 ml. of benzene-toluene (1 : 1) in the presence of 2 g. of granular activated zinc (Note 2). When the initial reaction subsides, the mixture is refluxed for 1 hour, then cooled and poured into crushed ice and 15 ml. of 2 *N* sulfuric acid. The aqueous phase is separated and extracted with four portions of benzene. The combined extracts are washed with three portions of 0.5 *N* sulfuric acid and water, then dried over sodium sulfate and concentrated; then the product is distilled at 58–60° (9 mm.). The radiochemical yield is 36% based on lithium acetate-1-C^{14} (Note 3).

(b) *3-Methylcrotonic-3-C^{14} Acid, (3-Methyl-2-butenoic-3-C^{14} Acid).* A mixture of 0.60 g. (4.12 mmoles) of ethyl 3-hydroxy-3-methylbutyrate-3-C^{14} and 4.12 mmoles of potassium bisulfate is heated under reflux for 3 hours at 160°. The ethyl 3-hydroxy-3-methylbutyrate-3-C^{14} is extracted into ether solution, which is then stored overnight with 4.5 mmoles of aqueous sodium hydroxide. The mixture is heated for 15 minutes on a steam-bath, and the ether is distilled. The aqueous solution is acidified with sulfuric acid, and the product is steam-distilled and crystallized from water; yield 50% (Note 4).

B. Notes

1. The procedure is a slight modification of the usual conditions[1] for the Reformatsky reaction.

2. A crystal of iodine is added at the start.

3. The acid is characterized as 3-hydroxy-3-methylbutyrhydrazide-3-C^{14},[2] m.p. 103°, formed by heating the ester with hydrazine hydrate in alcohol.

Sodium 3-hydroxy-3-methylbutyrate-3-C^{14} is formed by hydrolyzing the ester with an equivalent amount of sodium hydroxide in 50% alcohol at room temperature.

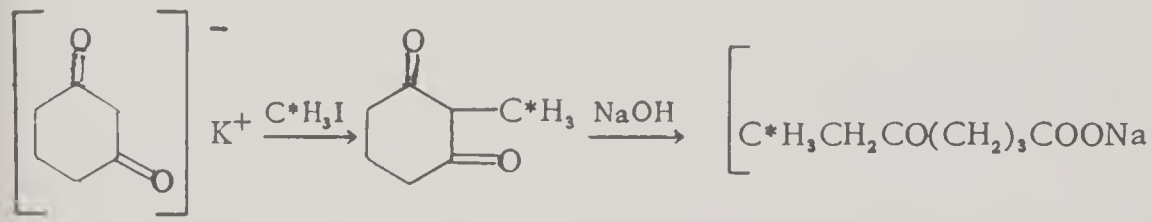
4. The Duclaux number is the same as that given by an authentic sample. Directions are given by Bloch for converting the acid with thionyl chloride to 3-methylcrotonoyl-3-C^{14} chloride (b.p. 150–151°) and 3-methylcrotonanilide-3-C^{14} (m.p. 127–128°), which was shown to be isotopically homogeneous by alumina chromatography.

Directions are also given for converting the C^{14}-acid chloride to the coenzyme A derivative.

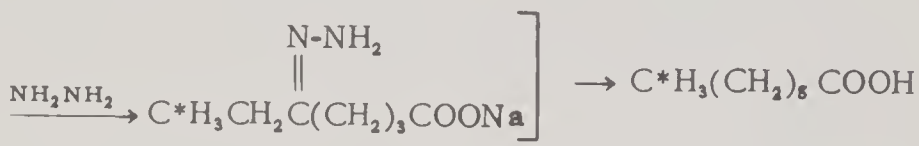
[1]*Organic Reactions*, Vol. I, Wiley, New York, 1942, Chapter 1.

[2]L. Ruzicka, G. Dalma, B. G. Engel and W. E. Scott, Helv. Chim. Acta, *24*, 1449 (1941).

HEPTANOIC-7-C^{14} ACID



(a)



(b)

R. M. Noller, Atomic Energy Commission Report, AECD-3504; Nuc. Sci. Abstracts, 7, 5013 (1953); Atomic Energy Commission Report, UCRL-2677 (1955).

A. Procedure (Note 1)

(a) *2-Methyl-C^{14}-1,3-cyclohexanedione, (2-Methyl-C^{14}-dihydroresorcinol).* A mixture of 2.21 g. (15.6 mmoles) of methyl-C^{14} iodide, 18.7 mmoles of potassium dihydroresorcinol[1] and a little water (Note 2) is heated for 4 hours at 65° in a sealed tube. The mixture is treated with aqueous sodium hydroxide solution (15.6 mmoles) and washed with ether to remove any methyl ethers and disubstitution products. The aqueous solution is adjusted to pH 5, and the insoluble product is collected, washed and dried; yield 45.1%, m.p. in the range 202–208° (Note 3).

(b) *Heptanoic-7-C^{14} Acid.* The crude 2-methyl-C^{14}-dihydroresorcinol is dissolved in methanol and treated with a pre-prepared reduction solution[2] containing 23.8 mmoles of sodium hydroxide, 15.2 ml. of diethylene

glycol and 0.95 ml. of 85% hydrazine hydrate. The constituents boiling below 150° are distilled off (Note 4), and the mixture is refluxed for 2 hours; then the components boiling below 195° are distilled off, and the residual solution is refluxed for 24 hours (Note 5). The mixture is cooled, acidified and extracted with benzene-ether (3 : 1). The extract, in turn is extracted with *N* sodium hydroxide solution; then the alkaline solution is acidified with 6 *N* sulfuric acid, and the product is steam-distilled. The distillate is titrated with standard sodium hydroxide solution (phenolphthalein); yield 39.6% based on methyl iodide (Note 6). The molar specific activity is the same as that of the starting material. Paper chromatography and radioautography indicate that there are no isotopic impurities (Note 7).

B. Notes

1. The procedure is a modification of the procedure of Stetter and Dierichs[3] for preparing long-chain acids.

2. The concentration of the solution may be varied over a considerable range without greatly affecting the yield. The optimum concentration is such that the salt melts below 65° but is solid at room temperature.

3. Refluxing for 30 hours in barium hydroxide solution opens the ring,[3] forming 5-oxoheptanoic acid in 78% yield, m.p. 50° from ether-ligroin.

4. A suction tube extending into the reflux condenser is used to draw off vapors.

5. Small portions of water may be added to maintain a stable reflux temperature.

6. The yield for this step is 85–90%.

7. A one-dimensional paper chromatogram is developed in propanol-ammonia-water (180 : 20 : 5 ml.). The known heptanoic acid spot is defined by a bromcresol green indicator spray.

[1]*Organic Syntheses*, Vol. 27, Wiley, New York, 1947, p. 21.
[2]*Organic Reactions*, Vol. IV, Wiley, New York, 1948, Chapt. 8.
[3]H. Stetter and W. Dierichs, Ber., *85*, 61 (1952).

DODECANOIC-1-C^{14} ACID
(Lauric-C^{14} Acid)

$$CH_3(CH_2)_{10}MgBr \xrightarrow{C^*O_2} CH_3(CH_2)_{10}C^*OOH$$

H. J. Harwood and A. W. Raltson, J. Org. Chem., *12*, 740 (1947).

A. Procedure

The carbon-C^{14} dioxide liberated from 0.5 g. (2.53 mmoles) of barium carbonate-C^{14}, by addition of concentrated sulfuric acid, is carried by a stream of dry nitrogen (25 cc./min.) into a stirred Grignard solution at −40°. The reagent is prepared from 14.1 g. (60 mmoles) of undecyl bromide, 1.5 g. (62.5 mmoles) of magnesium and 125 ml. of absolute ether under nitrogen. After 40 minutes, the flow of nitrogen is increased to 100 cc./min. for 10 minutes; then the reaction is completed by passing nonisotopic carbon dioxide (50–100 cc./min.) through the mixture, while the temperature is raised to 25°. The complex is hydrolyzed with dilute hydrochloric acid, and the ether solution is washed with water and evaporated to dryness. The residue is taken up in alcoholic potassium hydroxide and extracted with petroleum ether (Note 1). The aqueous solution is concentrated to remove organic solvents, then is acidified to precipitate the crude product, which is collected, washed with water and recrystallized from acetonitrile at −20°; yield 6.80 g. (f.p. 43.52°). A second crop (3.30 g.) is obtained by the use of 3.00 g. of carrier (Note 2), making the radiochemical yield over 95% (Note 3). The combined product freezes at 43.40° (Note 4).

Glyceryl dodecanoate-1-C^{14}, (C_3^{14}-trilaurin), is prepared in 71–80% yield[1] by heating together equivalent amounts of glycerol and the acid in an atmosphere of carbon dioxide, or under reduced pressure.[2]

B. Notes

1. The extraction with petroleum ether (b.p. 60–70°) removes undecane and docosane.

2. The combined mother liquors are evaporated to dryness, the residue (0.7 g.) is diluted with the carrier, and the mixture is recrystallized from 30 ml. of acetonitrile at −20°.

3. Geyer, Mathews and Stare[1] have reported isotopic yields of 75–86% by this method, m.p. 43.6–44.0°. The preparation also has been reported by Bloom,[3] and Jerchel[4] (60% yield, m.p. 43–44° at the 20.3-mmole scale).

4. A specimen, diluted with carrier and again recrystallized, melts at 44.2–44.7° (cor.). Lauric acid boils at 225° (100 mm.), n_D^{62} 1.4183.

[1]R. P. Geyer, L. W. Mathews and F. J. Stare, J. Biol. Chem., *180*, 1037 (1949).
[2]T. L. Garner, J. Soc. Chem. Ind., *47*, 278T (1928).
[3]B. Bloom, I. L. Chaikoff and W. O. Reinhardt, Am. J. Physiol., *166*, 451 (1951).
[4]D. Jerchel, H. Becker and K. Schmeiser, Z. Naturforsch., *9b*, 169 (1954).

TETRADECANOIC-1-C^{14} ACID
(Myristic-C^{14} Acid)

$$CH_3(CH_2)_{12}Br \xrightarrow{KC^*N} \underset{(a)}{CH_3(CH_2)_{12}C^*N} \xrightarrow{KOH} \underset{(b)}{CH_3(CH_2)_{12}C^*OOH}$$

H. S. Anker, J. Biol. Chem., *194*, 177 (1952).

A. Procedure (Note 1)

(a) *Tetradecanenitrile-1-C^{14}, (Myristonitrile-C^{14}).* A mixture of 2 mmoles of tridecyl bromide (Note 2), 1 mmole of potassium cyanide-C^{14} and 25 ml. of absolute alcohol is refluxed for several days (Note 3).

(b) *Tetradecanoic-1-C^{14} Acid.* The nitrile reaction mixture is refluxed with 80 ml. of 20% potassium hydroxide solution for 3 days. The mixture is extracted with ether; then the aqueous solution is acidified with hydrochloric acid and extracted with petroleum ether. The extract is dried and evaporated; yield 0.200 g. (87%), m.p. 52.0–53.5° (Note 4).

B. Notes

1. The procedure is adapted from the method of Chuit.[1] The isotopic preparation has been reported by Bergström.[2]

2. The starting material may be prepared by a procedure used to degrade fatty acids stepwise.[3] The silver salt of an acid is decarboxylated with bromine to the next lower alkyl bromide, which is hydrolyzed to the alcohol and oxidized to the corresponding acid. The preparation of myristic acid is described by Beal.[4]

3. Myristonitrile melts at 19° and boils at 226.5° (100 mm.).

4. The acid may be recrystallized[3] from petroleum ether (b.p. 40–60°). The highest recorded melting point in 53.8°, b.p. 199° (16 mm.). Directions are given by Bergström[2] for preparing the isotopic glyceride by transesterification.

C. Other Preparations

The preparation of myristic-C^{14} acid by carbonation of the Grignard reagent has been reported by Bloom[5] and by Gray[6] (yield 92% at the 3-mmole scale).

[1]P. Chuit, F. Boelsing, J. Hausser and G. Malet, Helv. Chim. Acta, *10*, 113 (1927).

[2]S. Bergström, B. Borgström and M. Rottenberg, Acta Physiol. Scand., *25*, 120 (1952).

[3]H. S. Anker, J. Biol. Chem., *194*, 177 (1952).

[4]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 379.

[5]B. Bloom, I. L. Chaikoff and W. O. Reinhardt, Am. J. Physiol., *166*, 451 (1951).

[6]I. Gray, Nucleonics, 7 (3), 58 (1950).

HEXADECANOIC-6-C^{14} ACID
(Palmitic-6-C^{14} Acid)

$$C_{10}H_{21}MgBr \xrightarrow{C^*O_2} \underset{(a)}{C_{10}H_{21}C^*OOH} \xrightarrow{CH_2N_2} \underset{(b)}{C_{10}H_{21}C^*OOCH_3} \xrightarrow[CuCr_2O_4]{H_2}$$

$$C_{10}H_{21}C^*H_2OH \xrightarrow{HBr} \underset{(c)}{C_{10}H_{21}C^*H_2Br} \xrightarrow{Mg} \underset{(d)}{C_{10}H_{21}C^*H_2MgBr} \xrightarrow{CdCl_2}$$

$$(C_{10}H_{21}C^*H_2)_2Cd \xrightarrow{CH_3OOC(CH_2)_3COCl} \underset{(e)}{C_{10}H_{21}C^*H_2CO(CH_2)_3COOCH_3} \xrightarrow{KOH}$$

$$\underset{(f)}{C_{10}H_{21}C^*H_2CO(CH_2)_3COOH} \xrightarrow{NH_2NH_2} \underset{(g)}{C_{10}H_{21}C^*H_2(CH_2)_4COOH}$$

W. G. Dauben, J. Am. Chem. Soc., *70*, 1376 (1948).

A. Procedure

(a) *Undecanoic-1-C^{14} Acid.* A Grignard solution (15.7 ml.) containing 11.7 mmoles of decylmagnesium bromide is carbonated with the carbon-C^{14} dioxide from 1.860 g. (9.42 mmoles) of barium carbonate-C^{14}, and the acid is isolated in the manner described for hexadecanoic-1-C^{14} acid (see benzoic-C^{14} acid).

(b) *Methyl Undecanoate-1-C^{14}.* The crude undecanoic-1-C^{14} acid is methylated with an ethereal solution of diazomethane. The solvent is evaporated, and the product is distilled in a small vacuum sublimation apparatus at 0.3 mm. and 54–58° (block temperature); yield 1.56 g., 82.9% based on barium carbonate, n_D^{23} 1.4275.

(c) *1-Undecanol-1-C^{14}* (Note 1). Methyl undecanoate-1-C^{14} (1.538 g., 7.67 mmoles) is hydrogenated over 1.0 g. of copper chromite[1] at 250° for 6 hours (3000 p.s.i. at room temperature, initially). The catalyst is filtered off, and the filtrate is refluxed for 1 hour with 10 ml. of 6 *N* sodium hydroxide and 5 ml. of methanol. The mixture is extracted with ether, and the extract is washed with water, dried over magnesium sulfate and concentrated.

(d) *1-Bromoundecane-1-C^{14}* (Note 2). The crude carbinol is halogenated by passage of anhydrous hydrogen bromide through the alcohol at 100° for about 15 minutes. The mixture is cooled, diluted with 15 ml. of hexane and washed with 2 ml. of cold concentrated sulfuric acid. The hexane solution is neutralized with dilute aqueous ammonia, then washed

with saturated sodium chloride, dried over magnesium sulfate and evaporated. The product is collected by distillation in a small sublimation-type still at 70–75° (block temperature) at 0.5 mm.; yield 1.432 g. (79.3%), n_D^{25} 1.4548.

(e) *Methyl 5-Oxohexadecanoate-6-C*^{14} (Note 3). To 0.15 g. (6.2 mmoles) of magnesium turnings, under nitrogen, is added slowly 1.412 g. (6.0 mmoles) of 1-bromoundecane-1-C^{14} in 50 ml. of absolute ether with stirring (Note 4). The Grignard solution is cooled with an ice-bath and treated with 0.71 g. (3.89 mmoles) of anhydrous cadmium chloride. The mixture is refluxed until a negative test[2] for Grignard reagent is obtained (2 hours). The solvent is replaced with benzene, the suspension is treated with 1.00 g. (6.1 mmoles) of 4-carbomethoxybutyryl chloride (Note 5), and the mixture is refluxed for 1 hour; then the solid mass is decomposed with acid.

(f) *5-Oxohexadecanoic-6-C*^{14} *Acid.* The ester is saponified by refluxing the reaction mixture with 0.4 g. of potassium hydroxide in 10 ml. of methanol. The mixture is diluted to 50 ml. with water and extracted with ether to remove 0.10 g. of docosane-11,12-C_2^{14} The alkaline solution is acidified and extracted with ether. The extract is washed with water and saturated sodium chloride, then dried over Drierite, and the solvent is evaporated (Note 6).

(g) *Hexadecanoic-6-C*^{14} *Acid.* A mixture of 6.4 ml. of diethylene glycol, 0.8 g. of sodium hydroxide, 0.77 ml. of 100% hydrazine hydrate and 0.89 g. of crude 5-oxohexadecanoic-6-C^{14} acid is heated for 1 hour at 150°, then for 4 hours at 220°. The warm mixture is acidified with dilute hydrochloric acid and extracted with ether. The extract is washed with water and saturated sodium chloride solution, then dried over magnesium sulfate and concentrated. The residue is distilled onto a cold finger at 1 mm. at 110° (bath temperature). The crude product is recrystallized from 15 ml. of 10% aqueous acetone, after decolorization with Norit; yield 0.700 g., m.p. 61–62°, 45.7% based on the bromide (Note 7). The molar specific activity is unchanged from that of the barium carbonate-C^{14}.

B. Notes

1. The procedure is a modification of the method of Adkins[3] for catalytically hydrogenating esters to alcohols. Three times the usual amount of catalyst is necessary to obtain consistently high yields, for the methanol produced destroys the activity.

2. The procedure is based on the method of Reid.[4]

3. The procedure is a modification of the method of Cason and Prout.[5] A review of the reaction between a dialkylcadmium compound and the ester chloride of a dibasic acid is presented by Cason.[6]

4. The halide is added over a period of 1.5 hours to minimize loss due to coupling.

5. The acid chloride is prepared by the method of Harris;[7] also see Cason.[8]

6. The keto acid is purified by recrystallization from methanol, m.p. 84.5–85°. The yield at the 2.86-mmole scale is 64% based on bromide (79% based on acid chloride).

7. In trial experiments, the yield in the Huang-Minlon modification of the Wolff-Kishner reduction[9] was 82%.

C. Other Preparations

By similar procedures are prepared hexadecanoic-5-C^{14} acid[10] and hexadecanoic-11-C^{14} acid.[11] The intermediates in the latter case are 1-bromohexane-1-C^{14}, dihexylcadmium-1-C_2^{14}, ethyl 10-oxohexadecanoate-11-C^{14} and 10-oxohexadecanoic-11-C^{14} acid (yield 18.6% based on bromide). The requisite 9-carbethoxynonanoyl chloride is prepared from ethyl hydrogen sebacate and thionyl chloride.

Hexadecanoic-13-C^{14} acid is prepared[12] from butyl-1-C^{14} bromide, employing 11-carbomethoxyhendecanoyl chloride.

Hexadecanoic-15-C^{14} acid is prepared[12] by the sequence: propionic-2-C^{14} acid, 1-propanol-2-C^{14}, 1-bromopropane-2-C^{14}, dipropylcadmium-2-C^{14} and 13-oxohexadecanoic-15-C^{14} acid. The requisite 12-carbomethoxydodecanoyl chloride is prepared by the half saponification[13] of methyl brassylate,[14] prepared from azelaic acid.[15]

Pentadecanoic-5-C^{14} acid is prepared[16] by substituting 3-carbomethoxypropionyl chloride for the corresponding butyryl derivative in the described procedure and reducing the methyl 4-oxopentadecanoate-5-C^{14}.

Octadecanoic-6-C^{14} acid is prepared[17] by condensing ditridecylcadmium-1-C_2^{14} with 4-carbomethoxybutyryl chloride and reducing the methyl 5-oxoöctadecanoate-6-C^{14}.

Octanoic-7-C^{14} acid is prepared [18,19,20] by condensing bis(ethyl-1-C^{14})-cadmium (prepared from ethylmagnesium-1-C^{14} bromide) with 5-carbethoxyvaleryl chloride,[21,17] forming ethyl 6-oxoöctanoate-7-C^{14}. Hydrolysis yields 6-oxoöctanoic-7-C^{14} acid, and reduction forms octanoic-7-C^{14} acid, which is purified by repeated steam-distillation and recrystallization from cold water, or by fractional distillation; over-all yield 68%, b.p. 108–109° (3 mm.). The S-benzylthiuronium salt melts at 149° (cor.).

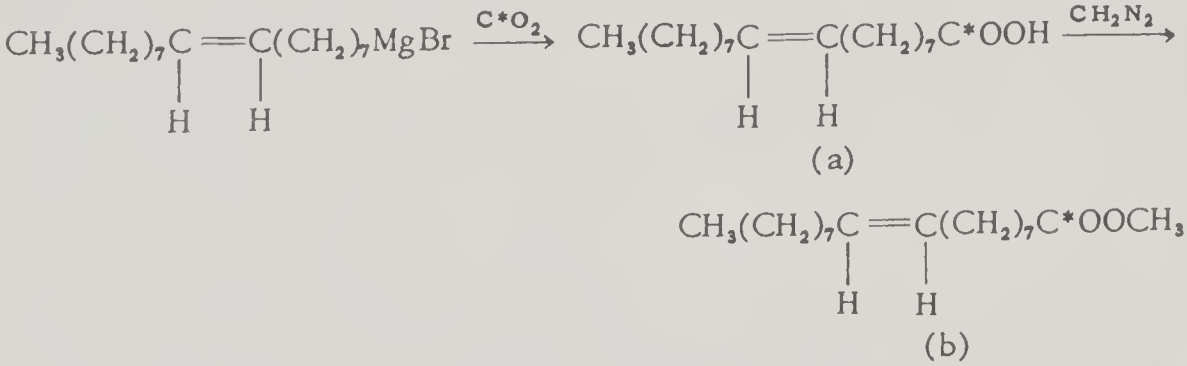
Methyl dodecanoate-1-C^{14} (92%, b.p. 140–141° at 15 mm.) and 1-bromododecane-1-C^{14} (79.6% b.p. 145° at 15 mm.) are prepared in a similar manner.[22]

[1] *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 142.
[2] H. Gilman and F. Schulze, J. Am. Chem. Soc., 47, 2002 (1925).
[3] H. Adkins and K. Folkers, J. Am. Chem. Soc., 53, 1095 (1931).

[4]*Organic Syntheses,* Coll. Vol. II, Wiley, New York, 1943, p. 246.
[5]J. Cason and F. S. Prout, J. Am. Chem. Soc., *66*, 46 (1944).
[6]J. Cason, Chem. Revs., *40*, 15 (1947).
[7]S. A. Harris, D. E. Wolf, R. Mozingo, G. E. Arth, R. C. Anderson, N. R. Easton and K. Folkers, J. Am. Chem. Soc., *67*, 2096 (1945).
[8]*Organic Syntheses,* Vol. 25, Wiley, New York, 1945, p. 19.
[9]Huang-Minlon, J. Am. Chem. Soc., *68*, 2487 (1946).
[10]I. L. Chaikoff, D. S. Goldman, G. W. Brown, Jr., W. G. Dauben and M. Gee, J. Biol. Chem., *190*, 229 (1951).
[11]E. O. Weinman, I. L. Chaikoff, W. G. Dauben, M. Gee and C. Entenman, J. Biol. Chem., *184*, 735 (1950).
[12]G. W. Brown, Jr., D. D. Chapman, H. R. Matheson, I. L. Chaikoff and W. G. Dauben, J. Biol. Chem., *209*, 537 (1954).
[13]R. Signer and P. Sprecher, Helv. Chim. Acta, *30*, 1001 (1947).
[14]P. Chuit, Helv. Chim. Acta, *9*, 264 (1926).
[15]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 53.
[16]I. L. Chaikoff, B. Bloom, B. P. Stevens, W. O. Reinhardt and W. G. Dauben, J. Biol. Chem., *190*, 431 (1951).
[17]E. O. Weinman, I. L. Chaikoff, B. P. Stevens and W. G. Dauben, J. Biol. Chem., *191*, 523 (1951).
[18]V. Lorber, M. Cook and J. Meyer, J. Biol. Chem., *181*, 475 (1949).
[19]D. D. Chapman, G. W. Brown, Jr., I. L. Chaikoff, W. G. Dauben and N. O. Fansah, Cancer Research, *14*, 372 (1954).
[20]D. I. Crandall, R. O. Brady and S. Gurin, J. Biol. Chem., *181*, 845 (1949).
[21]E. E. Blaise and A. Koehler, Bull. soc. chim. 7, 219 (1910).
[22]D. Jerchel, H. Becker and K. Schneiser, Z. Naturforsch., *9b*, 169 (1954).

OLEIC-1-C^{14} ACID
(*cis*-9-Octadecenoic-1-C^{14} Acid)

METHOD I



J. C. Nevenzel and D. R. Howton, Atomic Energy Commission Report, UCLA-356.

A. Procedure

(a) *Oleic-1-C^{14} Acid.* A Grignard solution containing 3.31 mmoles of *cis*-8-heptadecenylmagnesium bromide (Note 1) is carbonated with 3.78

mmoles of carbon-C^{14} dioxide in the manner described for the preparation of linoleic-1-C^{14} acid. The crude product is extracted into pentane solution, which is washed with water and evaporated, and the oil (0.98 g.) is chromatographed on a column of silicic acid. Fractional elution yields 0.185 g. of hydrocarbon (Note 2), 0.025 g. of material of intermediate polarity, 0.65 g. of product (neut. equiv. 280, theory 282) and 0.06 g. of more strongly absorbed material. The yield is 63.2% based on the bromide (Note 3).

(b) *Methyl Oleate-1-C^{14}.* Esterification is accomplished by treating an ether solution of the acid with diazomethane. Purification is accomplished by chromatography on a silicic acid column; yield 75% (Note 4).

METHOD II

$$CH_3(CH_2)_7CHOHCHOH(CH_2)_7Br \xrightarrow{KC^*N}$$

$$CH_3(CH_2)_7CHOHCHOH(CH_2)_7C^*N \xrightarrow{KOH}$$

$$CH_3(CH_2)_7CHOHCHOH(CH_2)_7C^*OOH \xrightarrow[H_2SO_4]{HBr\ HOAc}$$

(a)

$$CH_3(CH_2)_7\underset{\displaystyle Br}{\underset{|}{C}}H\underset{\displaystyle Br}{\underset{|}{C}}H(CH_2)_7C^*OOH \xrightarrow[H_2SO_4]{C_2H_5OH}$$

$$CH_3(CH_2)_7\underset{\displaystyle Br}{\underset{|}{C}}H\underset{\displaystyle Br}{\underset{|}{C}}H(CH_2)_7C^*OOC_2H_5 \xrightarrow{Zn}$$

$$CH_3(CH_2)_7\underset{\displaystyle H}{\underset{|}{C}}=\underset{\displaystyle H}{\underset{|}{C}}(CH_2)_7C^*OOC_2H_5 \xrightarrow{NaOH}$$

$$CH_3(CH_2)_7\underset{\displaystyle H}{\underset{|}{C}}=\underset{\displaystyle H}{\underset{|}{C}}(CH_2)_7C^*OOH$$

(b)

S. Bergström, K. Pääbo and M. Rottenberg, Acta Chem. Scand., 6, 1127 (1952).

A. Procedure

(a) erythro-9, *10-Dihydroxyoctadecanoic-1-C^{13} Acid.* A mixture of 2.8 mmoles of *erythro*-1-bromo-8,9-heptadecanediol (Note 5), 2.8 mmoles of

potassium cyanide-C^{13}, 10 ml. of methanol and 1 ml. of water is refluxed for 48 hours. The 9,10-dihydroxyoctadecanenitrile-1-C^{13} mixture is treated with 2 g. of potassium hydroxide and refluxed for a further 48 hours. The yield of product, melting at 126–127°, is 85% (Note 6).

(b) *Oleic-1-C^{13} Acid.* The yield of oleic-1-C^{13} acid is 2.5 g. (48%, m.p. 12°) from 5.0 g. of *erythro*-9,10-dihydroxyoctadecanoic-1-C^{13} acid. The preparation is by the procedure of Ames and Bowman,[1] which follows.

To 5 g. of *erythro*-9,10-dihydroxyoctadecanoic acid is added 50 ml. of an acetic acid solution of dry hydrogen bromide (d. 1.25), followed by 5 ml. of concentrated sulfuric acid in small portions with shaking and cooling. The solid dissolves in a few hours, and an oily layer separates in 16 hours; the mixture then is heated for 8 hours at 100°, with addition of a 5-ml. portion of the hydrogen bromide solution at 4 hours. The mixture is treated with water, and the oil is extracted into light petroleum (b.p. 60–80°). The extract is washed with water, dried over magnesium sulfate and concentrated (Note 7).

The residue of crude *threo*-9,10-dibromoöctadecanoic acid is esterified azeotropically with ethanol, sulfuric acid and benzene (Note 8). The crude ester is dissolved in 200 ml. of petroleum ether and passed through a column of activated alumina (6 in.), by washing with 400 ml. of the solvent. Distillation of the combined eluated yields 6.5 g. of ethyl *threo*-9,10-dibromoöctadecanoate, b.p. 210° at 0.3 mm., n_D^{20} 1.4866 (Note 9).

A mixture of 15 g. of zinc, 75 ml. of ethanol and 1 ml. of 60% aqueous hydrobromic acid is refluxed for 5 minutes (to activate the zinc), then treated with the dibromo ester and refluxed for 1 hour under nitrogen. The zinc is filtered off and washed with 100 ml. of light petroleum (b.p. 60–80°). The combined filtrates are washed with dilute sulfuric acid and water, then dried over magnesium sulfate, concentrated and vacuum-distilled (nitrogen leak). The yield of ethyl oleate is 3.4 g., b.p. 148° (0.4 mm.), n_D^{20} 1.4516, iodine value 81.9 (calc. 82.0, Note 3).

Saponification of the ester under nitrogen gives oleic acid, b.p. 160° (0.1 mm.), m.p. 12° n_D^{20} 1.4608, iodine value 90.0 (calc. 89.9).

B. Notes

1. The Grignard reagent is prepared in 83% yield. Detailed directions are given by Nevenzel and Howton for preparing the starting bromide, b.p. 134.0–136.5° (180 μ), n_D^{25} 1.4708. *threo*-9,10-Dibromoöctadecanoic acid, obtained by bromination of oleic acid, is converted to the silver salt and then to *threo*-1,8,9-tribromoheptadecane by bromine degradation. Following chromatographic separation, zinc-debromination gives *cis*-1-bromo-8-heptadecene, which is separated from small amounts of the less soluble *trans*-isomer by low-temperature crystallization from acetone. On

the basis of infrared absorption data, this material contains 10% *trans*-isomers.

2. Presumably 9,25-tetratricontadiene is formed by the Wurtz reaction.

3. The yield is 58.6% based on barium carbonate. The decarboxylation of oleic-1-C^{13} acid and higher fatty acids by the Schmidt reaction (yield 60–90%) is described by Blomstrand.[2]

4. The specific activity is 93% of the calculated value. The product contains 1.9% methyl linoleate, determined as *threo,threo*-9,10,12,13-tetrabromoöctadecanoic acid[3] by isotopic dilution technique. Specially purified commercial oleic acid (Note 1) is expected to contain about 3% each of linoleic and saturated acids.

5. Directions are given by Bergström for preparing the starting material from oleic acid. *erythro*-9,10-Dihydroxyoctadecanoic acid, m.p. 129°, prepared by the method of Lapworth,[4] is acetylated in acetic anhydride and pyridine. The dry silver salt of *erythro*-9,10-diacetoxyoctadecanoic acid is subjected to the silver salt bromine degradation.[5] The *erythro*-8,9-diacetoxy-1-bromoheptadecane is hydrolyzed in methanolic hydrogen chloride, yielding the *erythro*-1-bromo-8,9-heptadecanediol, m.p. 102–103°. See lithocholic-24-C^{14} acid and lineoleic-1-C^{14} acid, Note 2. A very simple procedure for preparing a similar silver salt is described by Howton.[6]

6. The method is similar to that of Noller[7] for synthesizing oleic acid. *erythro*-9,10-Dihydroxyoctadecanoic-1-C^{14} acid has been prepared in similar yields from an excess of the bromide.

7. The stereoisomerism involved in this method of preparing long chain olefins is discussed by Young.[8]

8. A method of esterification at the 10-mmole scale is described by Ames.[9]

9. There is some decomposition during distillation, if the chromatographic step is omitted.

[1]D. E. Ames and R. E. Bowman, J. Chem. Soc., *1951*, 1079.

[2]R. Blomstrand, Acta Chem. Scand., *8*, 1487 (1954).

[3]M. F. White and J. B. Brown, J. Am. Oil Chem. Soc., *26*, 385 (1949); Chem. Abstracts, *43*, 7721 (1949); *Organic Syntheses*, Vol. 22, Wiley, New York, 1942, p. 75.

[4]A. Lapworth and E. N. Mottram, J. Chem. Soc., *127*, 1628 (1925); A. R. Bader, J. Am. Chem. Soc., *70*, 3938 (1948); C. P. Hilditch, J. Chem. Soc., *1926*, 1828.

[5]M. Rottenberg, Helv. Chim. Acta, *35*, 1286 (1952); J. Kleinberg, Chem. Revs., *40*, 381 (1947).

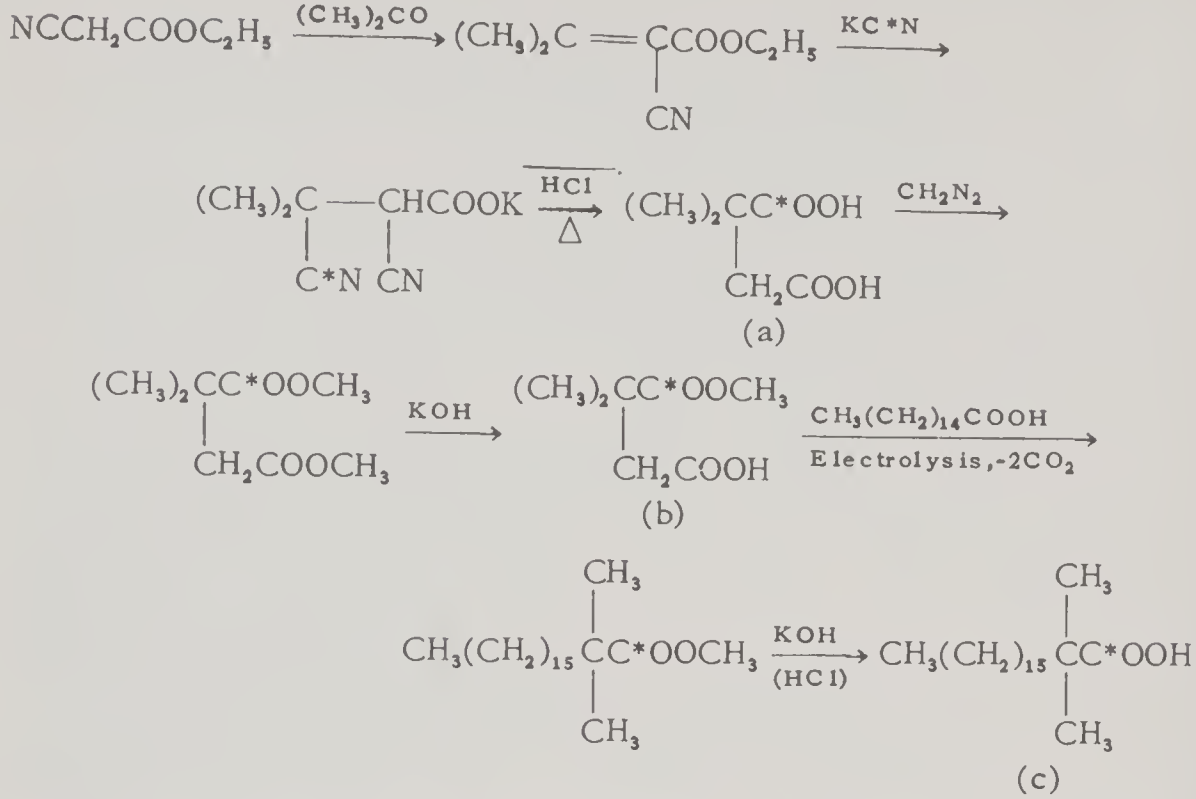
[6]D. R. Howton, R. H. Davis and J. C. Nevenzel, J. Am. Chem. Soc., *76*, 4970 (1954).

[7]C. R. Noller and R. A. Bannerot, J. Am. Chem. Soc., *56*, 1563 (1934).

[8]W. G. Young, Z. Jasaitis and L. Levanas, J. Am. Chem. Soc., *59*, 403 (1937).

[9]D. E. Ames and R. E. Bowman, J. Chem. Soc., *1951*, 1087.

2,2-DIMETHYLOCTADECANOIC-1-C^{14} ACID



S. Bergström, B. Borgström, N. Tryding and G. Westöö, Biochem. J., 58, 604 (1954).

A. Procedure

(a) *2,2-Dimethylsuccinic-1-C^{14} Acid* (Note 1). Solutions of 1.1 g. of ethyl 2-cyano-3-methylcrotonate in 5 ml. of ethanol and 0.55 g. of potassium cyanide-C^{14} in 1.25 ml. of water are mixed and stored at room temperature for 24 hours. The alcohol is distilled off, and the crude potassium 2-cyano-3-cyano-C^{14}-3-methylbutyrate is refluxed for 3 hours with a large excess of concentrated hydrochloric acid. The mixture is diluted with water, saturated with ammonium sulfate and extracted with 5 portions of ether. The extract is dried over sodium sulfate and evaporated. The product is purified by boiling the residue twice with small amounts of benzene, cooling and collecting the crystals at room temperature; yield 63%, m.p. 143° (Note 2).

(b) *3-Methoxycarbonyl-C^{14}-3-methylbutyric Acid, (Methyl Hydrogen C_1^{14}-2,2-Dimethylsuccinate)* (Note 3). 2,2-Dimethylsuccinic-1-C^{14} acid (11.0 g., 75.3 mmoles) is dissolved in methanol and esterified with diazomethane in absolute ether. The solvents are distilled, and the methyl 2,2-dimethylsuccinate-1-C^{14} is converted into the half-ester by hydrolysis with 45.1 ml. of 1.67 *N* methanolic potassium hydroxide and 5 ml. of water. The solution is stored overnight, refluxed for 3–4 hours,

diluted with water, acidified with hydrochloric acid and extracted with ether. The extract is dried over sodium sulfate and evaporated under vacuum; yield 11.5 g. (Note 4).

(c) *2,2-Dimethyloctadecanoic-1-C^{14} Acid* (Note 5). An ice-cooled mixture of 0.45 g. of sodium in 300 ml. of methanol, 5.7 g. of crude 3-methoxycarbonyl-C^{14}-3-methylbutyric acid and 20 g. of palmitic acid is electrolyzed with platinum electrodes and a current density of 0.3 amp. per sq. cm., methanol being added to replace that lost by evaporation. When the mixture becomes alkaline to litmus, another 5.7 g. of the half-ester and 20 g. of palmitic acid are added, and the reaction is continued. The slightly alkaline solution is cooled, and the precipitated triacontane (Note 6) is filtered off and extracted once with boiling methanol (cooled before being filtered). The combined solution is evaporated, and the residue is extracted into ether solution, which is washed with sodium carbonate solution until no more palmitate precipitates. The solution is filtered, washed with water and evaporated. The residual methyl 2,2-dimethyloctadecanoate-1-C^{14} is hydrolyzed by heating for 6 hours at 120° (autoclave) with 25 g. of potassium hydroxide, 10 ml. of water and 170 ml. of ethanol. The hydrolysate is diluted with 150 ml. of water and washed with 2 portions of petroleum ether, then is acidified with hydrochloric acid and extracted with ether. The extract is dried over sodium sulfate and evaporated; yield 2 g. Another 7 g. of crude product is obtained by extracting the petroleum ether with 50% (w/v) alcohol, acidifying the aqueous solution and extracting with ether. The combined product is recrystallized from methanol; yield 6.8 g. (29% based on 2,2-dimethylsuccinic acid), m.p 57° (Note 7).

B. Notes

1. The procedure is a slight modification of the method of Vogel.[1]

2. Neutralization equivalent 73.1, calc. 73.1.

3. The procedure is an adaptation of that of Bone[2] for preparing the acid ester from the normal ester.

4. Methyl 2,2-dimethylsuccinate boils at 201–202°. 3-Methoxycarbonyl-3-methylbutyric acid melts at 40.5°, b.p. 141° (14 mm.); the isomeric acid melts at 52°.[2]

5. The procedure is an adaptation of the method of Stăllberg-Stenhagen[3] for chain lengthening by the electrolysis of sodium salts of carboxylic acids. A review of anodic syntheses with carboxylic acids, the Kolbe reaction, is presented by Weedon.[4]

6. Triacontane, prepared by electrolysis of sodium palmitate and crystallized from nitrobenzene, melts at 66.8°.

7. Recrystallization from ligroin raises the melting point to 58.5°.

C. Other Preparations

3α,7α,12α-Coprostanetriol-4-C^{14} has been prepared[5] by electrolysis of a mixture of cholic-4-C^{14} acid and a large excess of 3-methylbutyric acid; m.p. 186°, $[\alpha]_D^{20}$ 15.1° ± 2° (0.9 ethanol).

2,2,17,17-Tetramethyloctadecanoic-1-C^{14} acid has been prepared[6] from 15,15-dimethylhexadecanoic acid and 3-methoxycarbonyl-C^{14}-3-methylbutyric acid by anodic synthesis; m.p. 46.6–46.8°, equiv. wt. 341 (calc. 340.6).

2-Methyloctadecanoic-1-C^{14} acid has been similarly prepared.[6]

[1]I. Vogel, J. Chem. Soc., *1928*, 2019, 2020.
[2]W. A. Bone, J. J. Sudborough and C. H. G. Sprankling, J. Chem. Soc., *85*, 534 (1904).
[3]S. Ställberg-Stenhagen, Arkiv. Kemi, *2*, 436 (1950); *3*, *ibid.*, 278 (1951).
[4]B. C. L. Weedon, Quart. Revs. (London), *VI (4)*, 380 (1952).
[5]S. Bergström, K. Pääbo and J. A. Rumpf, Acta Chem. Scand., *8*, 1109 (1954).
[6]N. Tryding and G. Westöö, Acta Chem. Scand., *8*, 1110 (1954).

PHENYLACETIC-1-C^{14} ACID

METHOD I

$$C_6H_5CH_2MgCl \xrightarrow{C^*O_2} C_6H_5CH_2C^*OOH$$

W. G. Dauben, J. C. Reid, P. E. Yankwich and M. Calvin, J. Am. Chem. Soc., *72*, 121 (1950).

A. Procedure

Benzylmagnesium chloride is carbonated with carbon-C^{14} dioxide in the manner described for preparing benzoic-C^{14} acid. The yield is 88.3% (m.p. 75.5–76.5°) at the 43-mmole scale.

METHOD II

$$C_6H_5C^*OCl \xrightarrow{CH_2N_2} \underset{(a)}{C_6H_5C^*OCHN_2} \xrightarrow{Ag_2O} C_6H_5CH{=}C^*O \xrightarrow{CH_3OH}$$

$$C_6H_5CH_2C^*OOCH_3 \xrightarrow{KOH} C_6H_5CH_2C^*OOH$$
$$\underset{(b)}{\qquad\qquad} \qquad \underset{(c)}{\qquad}$$

W. G. Dauben, J. C. Reid, P. E. Yankwich and M. Calvin, J. Am. Chem. Soc., *72*, 121 (1950).

A. Procedure (Note 1)

(a) *2-Diazoacetophenone-1-C^{14}*. A solution of 1.26 g. (8 mmoles) of benzoyl-C^{14} chloride in 5 ml. of absolute ether is added slowly to a

solution of 1.15 g. (26 mmoles) of diazomethane in 78 ml. of absolute ether maintained at 0°. After 3 hours, the mixture is warmed to room temperature and evaporated to dryness under reduced pressure (Note 2).

(b) *Methyl Phenylacetate-1-C^{14}* (Note 3). To a solution of the 2-diazoacetophenone-1-C^{14} in 20 ml. of methanol at 70° is added 0.050 g. of silver oxide (Note 4) in portions over 6 hours; then the mixture is refluxed for 6 hours. The catalyst is removed by centrifugation.

(c) *Phenylacetic-1-C^{14} Acid.* The methyl phenylacetate-1-C^{14} is saponified by treating the methanolic solution with 10 ml. of 20% potassium hydroxide and boiling off the alcohol. The alkaline mixture is washed with ether, then acidified to precipitate the product. The yield of purified acid, recrystallized from water, is 47%, m.p. 74–76°. Degradation by the Hofmann reaction shows that the isotope is located in the carboxyl group exclusively.

B. Notes

1. The synthesis is incidental to a study of the mechanism[1,2] of the Arndt-Eistert[2,3] procedure for converting an acid to its next higher homolog. An excellent discussion is presented by Bachmann and Struve.[4]

2. The product, purified by trituration and crystallization from light petroleum, forms pale-yellow, flat needles, m.p. 48–48.5°; Bradley and Robinson[5] report a 91% yield. If diazomethane is not in excess at all times, the product formed is 2-chloroacetophenone[6] (72% yield).

2-Diazoacetophenone-1-C^{14} has been converted, to 4-pentyl-1-phenacyl-β-C^{14}-pyridinium *p*-toluenesulfonate by treatment with 4-pentylpyridinium *p*-toluenesulfonate; yield 46% based on barium carbonate.

3. This phase of the Arndt-Eistert synthesis is the Wolff rearrangement.[8] The catalyst is believed to accelerate the decomposition of the diazoketone to the ketene, for in its absence no rearrangement occurs, and the product, in the presence of water, is 2-hydroxyacetophenone instead of phenylacetic acid.

4. The catalyst may be prepared by treating a 10% solution of silver nitrate with dilute sodium hydroxide solution until precipitation is complete, but avoiding an excess of alkali. The precipitate is washed well by decantation and filtration.

C. Other Preparations

Phenylacetic-1-C^{13} acid has been prepared by Huggett,[1] in a study of the mechanism of the Arndt-Eistert reaction. The 2-diazoacetophenone-1-C^{13} was decomposed and rearranged in aqueous mixture, thereby eliminating the ester saponification step of Method II. Degradation, by decarboxylation with copper chromite and quinoline, showed that the

isotope is found in the new carboxyl group exclusively, thus supporting the ketene mechanism.[2]

The preparation of phenylacetic-1-C^{14} acid by Method I has been reported by Lee,[9] Arnstein[1]; and Sebek;[11] Vaughan[14] has described in detail a similar procedure (93.4% at the 500-mmole level), in which carbonation is carried out at $-50°$.

Phenylacetic-1-C^{14} acid has been prepared quantitatively[13] by hydrolysis of 2-phenylacetanilide-1-C^{14} with a mixture of acetic and hydrochloric acids in a sealed tube below 200° for 8 hours. Degradation by chromic acid oxidation formed nonisotopic benzoic acid.

For the preparation of 2-diazoacetophenone-1-C^{13} by another method, see mandelic-α-C^{13} acid, Other Preparations.

[1]C. Huggett, R. T. Arnold and T. I. Taylor, J. Am. Chem. Soc., *64*, 3043 (1942).
[2]B. Eistert, Ber., *68*, 208 (1935).
[3]F. Arndt and B. Eistert, Ber., *68*, 200 (1935).
[4]*Organic Reactions*, Vol. I, Wiley, New York, 1942, Chapter 2.
[5]W. Bradley and R. Robinson, J. Chem. Soc., *1928*, 1310.
[6]D. A. Clibbens and M. Nierenstein, J. Chem. Soc., *107*, 1491 (1915).
[7]R. G. Pearson and P. Sellers, Nucleonics, 7 (4), 48 (1950).
[8]L. Wolff, Ann., *394*, 23 (1912).
[9]C. C. Lee and J. W. T. Spinks, Can. J. Chem., *31*, 761 (1953).
[10]H. R. V. Arnstein, M. Clubb and P. T. Grant, *Radioisotope Conference 1954*, Vol. I, Academic Press, New York, 1954, p. 306.
[11]O. K. Sebek, Proc. Soc. Exptl. Biol. Med., *84*, 170 (1953).
[12]W. R. Vaughan and D. I. McCane, J. Am. Chem. Soc., *76*, 2504 (1954). J. A. Mitchell and E. E. Reid, J. Am. Chem. Soc., *53*, 321 (1931).
[13]C. J. Collins and O. K. Neville, J. Am. Chem. Soc., *73*, 2471 (1951).

PHENYLACETIC-2-C^{14} Acid

$$C_6H_5C^*OOH \xrightarrow{CH_2N_2} C_6H_5C^*OOCH_3 \xrightarrow{LiAlH_4} C_6H_5C^*H_2OH \xrightarrow{SOCl_2}$$
(a) (b)

$$C_6H_5C^*H_2Cl \xrightarrow[2.\ CO_2]{1.\ Mg} C_6H_5C^*H_2COOH$$
(c) (d)

J. A. Montgomery and R. E. Burks, Jr., Southern Research Institute, Birmingham, Ala., private communication.

A. Procedure

(a) *Methyl Benzoate-C^{14}*. An ether solution of 1.952 g. (16 mmoles) of benzoic-C^{14} acid is treated with a cold etheral solution of diazomethane

prepared from 10 g. of 1-nitroso-1-methylurea.[1] The excess diazomethane and ether are distilled off.

(b) *Benzyl-α-C^{14} Alcohol* (Note 1). An ether solution of the ester is added dropwise tc a stirred ether solution of 1.82 g. (48 mmoles) of lithium aluminum hydride, under anhydrous conditions, at such a rate as to maintain gentle boiling. The mixture then is refluxed for 2 hours, cooled with an ice-bath and acidified with dilute hydrochloric acid. The aqueous layer is separated and extracted with several portions of ether, and the combined extract is dried over magnesium sulfate and concentrated.

(c) *α-Chlorotoluene-α-C^{14}, (Benzyl-α-C^{14} Chloride)*. To the carbinol is added slowly 5 ml. of purified thionyl chloride, and the mixture is refluxed for 1 hour. The excess thionyl chloride is distilled off, and the product is collected by distillation at reduced pressure. The yield is 1.75 g. of a pale yellow oil, b.p. 81–83° (27 mm.), 86.3% based on benzoic acid (Note 2).

(d) *Phenylacetic-2-C^{14} Acid.* To a stirred mixture of 0.34 g. (14 mmoles) of magnesium turnings and absolute ether is added an ether solution of the benzyl-α-C^{14} chloride at a rate to cause gentle boiling. The mixture is refluxed for 1 hour, cooled to –20° and carbonated by passing in dry carbon dioxide gas with vigorous stirring. The Grignard complex is destroyed by the careful addition of dilute hydrochloric acid, and the acid layer is separated and extracted with several portions of ether. The combined ether solution is extracted with dilute sodium hydroxide, and the alkaline solution is acidified with cooling to precipitate the crude product, which is collected and dried; yield 1.65 g. (Note 3). Degradation studies indicate that there is no isotopic rearrangement (Note 4).

B. Notes

1. The procedure is adapted from the method of Nystrom and Brown.[2] Dauben[3] has obtained a yield of 85.5% (b.p. 114–115° at 32 mm.) based on 229 mmoles of the ester.

2. A yield of 94.4% at this step has been reported by Reid.[4] A mixture of 0.481 g. of benzyl-α-C^{14} alcohol, 5 mmoles of thionyl chloride, 0.01 ml. of dry pyridine and 5 ml. of absolute ether is refluxed for 1 hour. The mixture is diluted with 5 ml. of ether, and the pyridine hydrochloride is separated by centrifuging. The solution is concentrated and the product is distilled; yield 0.539 g., bath temperature 120° (39 mm.), The compound tests sulfur-free, b.p. 177° (Siwoloboff).

3. Purification may be effected by recrystallization from petroleum ether, m.p. 76–77°. The molar specific activity is the same as that of the starting ester.[3]

4. Directions are given by Dauben[3] for decarboxylating the acids under various conditions. With a commercial copper chromite catalyst in quinoline at temperatures of 170°, 205° and 230°, the evolved carbon dioxide contained 0.07, 0.5 and 0.6% of the initial activity, respectively. The middle figure dropped to 0.3%, when the copper chromite was prepared according to Lazier and Arnold,[5] and to 0.1% when the catalyst was first reduced with hydrogen. No trace of activity was detected when the decarboxylation was effected with copper or iron powder. The carbon dioxide evolved in the decarboxylation is diluted with some formed by partial oxidation of the rest of the molecule by the small amounts of cupric oxide in the copper chromite catalyst. The carbon-C^{14} dioxide evolved from phenylacetic-1-C^{14} acid, in this manner, was found[6] to possess a molar specific activity 3% less than that of the starting material; whereas degradation by the Hofmann reaction (through the amide) formed nonisotopic benzylamine. Ambiguous results have also been reported by Brown.[7]

C. Other Preparations

By similar procedures, starting with phenylacetic-2-C^{14} acid, are prepared:[3]

Phenethyl-β-C^{14} alcohol, 85.6%, b.p. 114° (9 mm.)
(2-Chloroethyl-2-C^{14})benzene, 33.8%, b.p. 55° (2 mm.).
Hydrocinnamic-β-C^{14} acid, (3-Phenylpropionic-3-C^{14} acid), 51.5%, m.p. 48–49°.

The carbon dioxide evolved by heating 3-phenylpropionic-3-C^{14} acid over copper chromite in quinoline at 230° contained 0.3% of the initial activity; see Note 4.

The preparation of benzyl-α-C^{14} alcohol by reduction of benzoic-C^{14} acid with lithium aluminum hydride has been reported by Neville[8] and Wheland,[9] and described by Bergel,[10] Pines[11] (yield 76%, b.p. 90.0–90.5° at 11 mm., n_D^{20} 1.5398) and Reid (71.5% yield, 85% in trial preparations).[4] The preparation by electroreduction of the acid has been described by Bills (80% yield; see toluene-2-C^{14}).

Benzyl-α-C^{14} chloride has been prepared by Dauben,[3] in 88.1% yield (b.p. 60–61° at 12 mm.) from benzyl-α-C^{14} alcohol, by treatment with phosphorus trichloride at the 195-mmole level. Bergel[10] has accomplished the chlorination by treating 3.5 mmoles of benzyl-α-C^{14} alcohol with 2 ml. of a solution of 7 g. of anhydrous zinc chloride in 5 ml. of concentrated hydrochloric acid. After standing overnight, the mixture was extracted with light petroleum, and the extract was washed with sodium carbonate solution and filtered through sodium sulfate.

Phenylacetic-2-C^{14} acid has been prepared by Dauben[3] (74% yield) by carbonating the Grignard reagent at -5°.

Phenylacetic-2-C^{14} acid has been prepared by Dauben[6] and Brown,[7] by alkaline hydrolysis of 2-phenylacetamide-2-C^{14}.

The preparation of phenylacetic-2-C^{13} acid from acetophenone-1-C^{13} by the Kindler-Willgerodt reaction (morpholine), followed by alkaline hydrolysis of the thiomorpholide, has been described by Schantz.[12] The preparation of phenylacetic-2-C^{14} acid (23% yield), by the same procedure, has been reported by Dauben.[6]

The formation of phenylacetic-2-C^{14} acid by the Willegrodt reaction (1.5–2%) has been reported by Dauben[6] and Brown.[7]

α-Bromotoluene-1,3,5-C^{14}_3 has been prepared[13] in 71% yield by bromination[14] of toluene-1,3,5-C^{14}_3. To a stirred solution of toluene and C. P. carbon tetrachloride, in a bath at 57° and illuminated by two 300-watt clear-glass Mazda lamps, is added slowly a slight excess of bromine in carbon tetrachloride. The solution is washed with water, dried and fractionally distilled. Benzyl bromide boils at 210°.

[1]*Organic Syntheses,* Coll. Vol. II, Wiley, New York, 1943, p. 165.

[2]R. F. Nystrom and W. G. Brown, J. Am. Chem. Soc., *69,* 1197 (1947). *Organic Reactions,* Vol. VI, Wiley, New York, 1951, Chapter 10. A. E. Finbolt, A. C. Bond, Jr., and H. I. Schlesinger, J. Am. Chem. Soc., *69,* 1199 (1947).

[3]*W. G.* Dauben and P. Coad, J. Am. Chem. Soc., *71,* 2928 (1949).

[4]J. C. Reid, Atomic Energy Commission Report, AECU-216; Nuc. Sci. Abstracts, *2,* 2091 (1949). P. Carre and D. Libermann, Compt. rend., *198,* 274 (1934).

[5]*Organic Syntheses,* Coll. Vol. II, Wiley, New York, 1943, p. 142.

[6]W. G. Dauben, J. C. Reid, P. E. Yankwich and M. Calvin, J. Am. Chem. Soc., *72,* 121 (1950).

[7]E. V. Brown, E. Cerwonka and R. C. Anderson, J. Am. Chem. Soc., *73,* 3735 (1951).

[8]O. K. Neville, Nucleonics, 7 (4), 45 (1950).

[9]G. W. Wheland and J. R. Schwartz, J. Chem. Phys., *17,* 425 (1949).

[10]F. Bergel, V. C. E. Burnop and J. A. Stock, J. Chem. Soc., *1955,* 1223.

[11]H. Pines and R. W. Myerholtz, Jr., J. Am. Chem. Soc., *77,* 5392 (1955).

[12]E. M. Schantz and D. Rittenberg, J. Am. Chem. Soc., *68,* 2109 (1946).

[13]B. Schepartz and S. Gurin, J. Biol. Chem., *180,* 663 (1949).

[14]J. R. Sampey, F. S. Fawcett and B. A. Morehead, J. Am. Chem. Soc., *62,* 1839 (1940).

CINNAMIC-α-C^{14} ACID

$$C^*H_2(COOH)_2 \xrightarrow{C_6H_5CHO} C_6H_5CH=C^*(COOH)_2 \longrightarrow C_6H_5CH=C^*HCOOH$$

J. C. Bevington, G. M. Guzman and H. W. Melville, Proc. Roy. Soc. (London), *221A,* 453 (1954).

A. Procedure (Note 1)

To a mixture of 5 ml. of freshly distilled benzaldehyde, 5.7 g. of malonic-2-C^{14} acid, 5 ml. of pyridine and 1 g. of anhydrous sodium sulfate is added 0.25 ml. of piperidine. The mixture is heated under reflux for 2 hours at 100°, then at 120° for 2 hours and finally poured into 250 ml. of ice water. The product is precipitated by the addition of 13 ml. of concentrated hydrochloric acid, collected, washed with cold water, and recrystallized from water; yield 75% (Note 2).

B. Notes

1. The procedure is an adaptation of the method of Dalal[1,2] for synthesizing α-unsaturated acids. The method appears to be more convenient than the Perkin synthesis.

2. Cinnamic acid melts at 131.5–132°; distillation at 146° results in partial decarboxylation to styrene.

C. Other Preparations

In a similar manner is prepared[3] cinnamic-β-C^{14} acid from benzaldehyde-C^{14}; over-all yield 40% based on benzoic-C^{14} acid.

Cinnamic-α-C^{14} acid has been prepared by Bevington and by Ropp[4] by means of the Perkin reaction,[2] employing benzaldehyde, sodium acetate-2-C^{14} and acetic anhydride. Sodium acetate-1-C^{14} yields cinnamic-C^{14} acid.

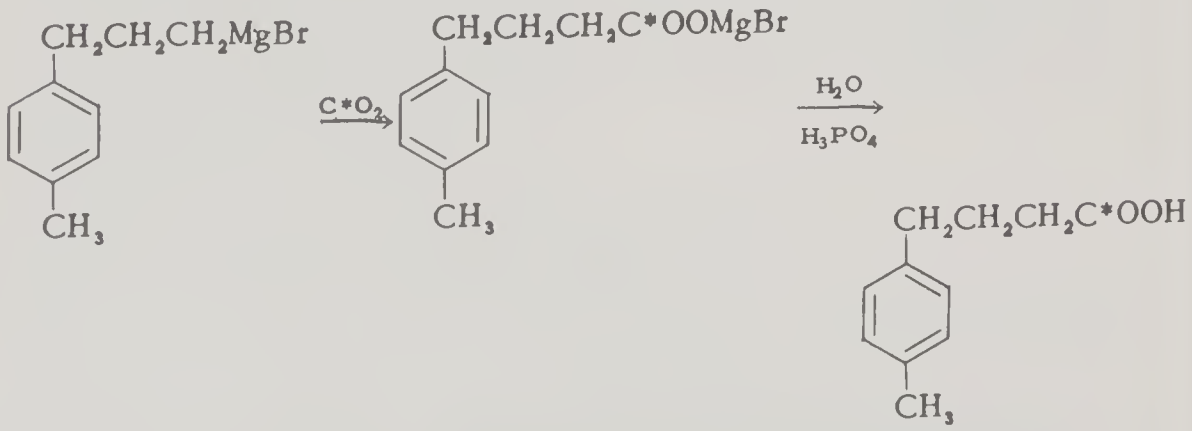
[1]M. Dalal and S. Dutt, J. Indian Chem. Soc., 9, 309 (1932); Chem. Abstracts, 27, 279 (1933).

[2]*Organic Reactions*, Vol. I, Wiley, New York, 1942, p. 248.

[3]S. A. Brown and A. C. Neish, Can. J. Biochem. and Physiol., 33, 948, (1955).

[4]G. A. Ropp, V. F. Raaen and A. J. Weinberger, J. Am. Chem. Soc., 75, 3694 (1953).

4-*p*-TOLYLBUTYRIC-1-C^{14} ACID



C. J. Collins, J. Am. Chem. Soc., 73, 1038 (1951).

A. Procedure

A Grignard reagent is prepared by refluxing for 1 hour, in an atmosphere of dry helium (Note 1), a stirred mixture of 0.90 g. of magnesium turnings, 25 ml. of absolute ether and 6.37 g. (5.0 ml.) of 3-*p*-tolylpropyl bromide (Note 2). The Grignard solution is cooled in a bath at −40 to −45° (Note 3) and treated with the anhydrous carbon-C^{14} dioxide (Note 4) liberated slowly (0.5 hour) from 6.00 g. of barium carbonate-C^{14} (Note 5) and swept into the reaction mixture by a gentle stream of helium. The Grignard addition product begins to precipitate almost immediately (Note 6). After an additional 5-minute period of sweeping, the complex is decomposed by the slow addition of 50 ml. of an aqueous solution containing 7 ml. of phosphoric acid, while the freezing bath is removed, the stirred mixture is slowly warmed to room temperature, and the gas sweeping is continued for 1 hour. The product is extracted continuously into ether for 4 hours (Note 7). The extract is concentrated, and the crystallized oil is dissolved by stirring with 75 ml. of saturated sodium bicarbonate solution for 1 hour. The alkaline solution is washed with 3 to 5 ml. portions of ether (Note 8), then acidified with 20 ml. of concentrated hydrochloric acid, and the precipitated product is collected by continuous ether extraction. Evaporation and desiccation yield 2.60 g. of product, m.p. 51–55°, 85.5% based on unrecovered barium carbonate (Note 9). Recrystallization from petroleum ether gives colorless plates melting at 57.5–59°.

B. Notes

1. A diagram and detailed description of the operation of an all-glass Grignard carbonation apparatus, designed for high activity syntheses, are presented by Collins. The system is swept with dry helium, and a positive pressure is maintained throughout its use. Unreacted carbon-C^{14} dioxide is trapped in a bubbler containing a half-saturated barium hydroxide solution. The reflux condenser is cooled with a powdered Dry Ice-chloroform-carbon tetrachloride mixture, and stirring is done with a magnetic bar.

2. Directions are given by Collins for preparing the starting material by reduction of 3-*p*-tolylpropionic acid with lithium aluminum hydride, followed by halogenation of the carbinol with hydrobromic acid.

3. The reagent precipitates at a lower temperature. The yield of carboxylic acid is reduced by carbonation at a higher temperature, the main product being nonacidic when temperatures above −20° are employed.

4. The gas is dried by passage through a spiral trap cooled with Dry Ice.

5. In this case, carbon dioxide was liberated by addition of 5 *N* perchloric acid to a stirred aqueous suspension of barium carbonate. Concentrated sulfuric acid is often preferable for this purpose.

6. The precipitate dissolves if the bath temperature rises to $-20°$.

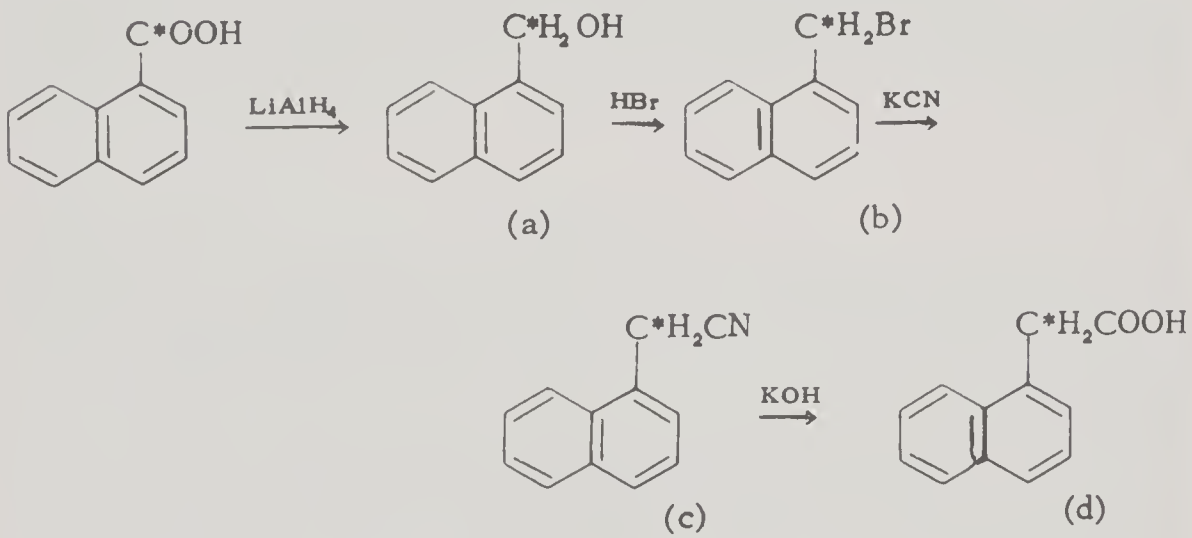
7. A continuous extractor is an integral part of the system described by Collins.

8. An extraction apparatus, designed to prevent mechanical losses and minimize laboratory contamination with high level syntheses, is described by Collins.[1]

9. The yield was 90–96% in several model runs. The barium carbonate-C^{14} (2.62 g.) is recovered from the bubbler, following aeration with carbon dioxide-free air for 4 hours while being warmed with an infrared lamp.

[1]C. J. Collins, J. Am. Chem. Soc., *70*, 2418 (1948).

1-NAPHTHALENEACETIC-α-C^{14} ACID



P. T. Adams and D. Kritchevsky, Atomic Energy Commission Report, UCRL-1262; Nuc. Sci. Abstracts, *5*, 4723 (1951).

A. Procedure (Note 1)

(a) *1-Naphthalenemethanol-α-C^{14}.* The reduction of 0.400 g. of 1-naphthoic-C^{14} acid is achieved with an excess of lithium aluminum hydride in ether, according to the procedure of Nystrom and Brown[1] (Note 2).

(b) *1-(Bromomethyl-C^{14})naphthalene* (Note 3). A dry ethereal solution of the carbinol is treated with dry, gaseous hydrogen bromide (Note 4). After 30 minutes, the mixture is treated with water, and the aqueous layer is separated and extracted with ether. The combined ether solution is dried over potassium carbonate, filtered and concentrated, leaving a heavy, yellow oil (Note 5).

(c) *1-Naphthaleneacetonitrile-α-C^{14}*. To a solution of the crude 1-(bromomethyl-C^{14})naphthalene in 10 ml. of alcohol is added 0.425 g. (6 mmoles) of potassium cyanide in 5 ml. of water and a crystal of potassium iodide. The mixture is refluxed for 18 hours; then the alcohol is evaporated, the dilute aqueous solution is extracted with ether, and the extract is dried and concentrated (Note 6).

(d) *1-Naphthaleneacetic-α-C^{14} Acid*. The crude nitrile is hydrolyzed by refluxing for 24 hours with 10 ml. of 10% potassium hydroxide solution. The product is isolated by acidification and ether extraction of the aqueous solution. Crystallization from hot water yields 0.151 g. of colorless needles, m.p. 130–131° (35% based on barium carbonate).

B. Notes

1. The method is a modification of the method of Cambron[2] for preparing 1-naphthaleneacetic acid; also see Manske.[3]

2. 1-Naphthalenemethanol crystallizes from water as needles, m.p. 59.5–60°; b.p. 301° (715 mm.), 163–166° (15 mm.) and 143–145° (2 mm.).

3. The procedure is according to the method of Pommereau.[4]

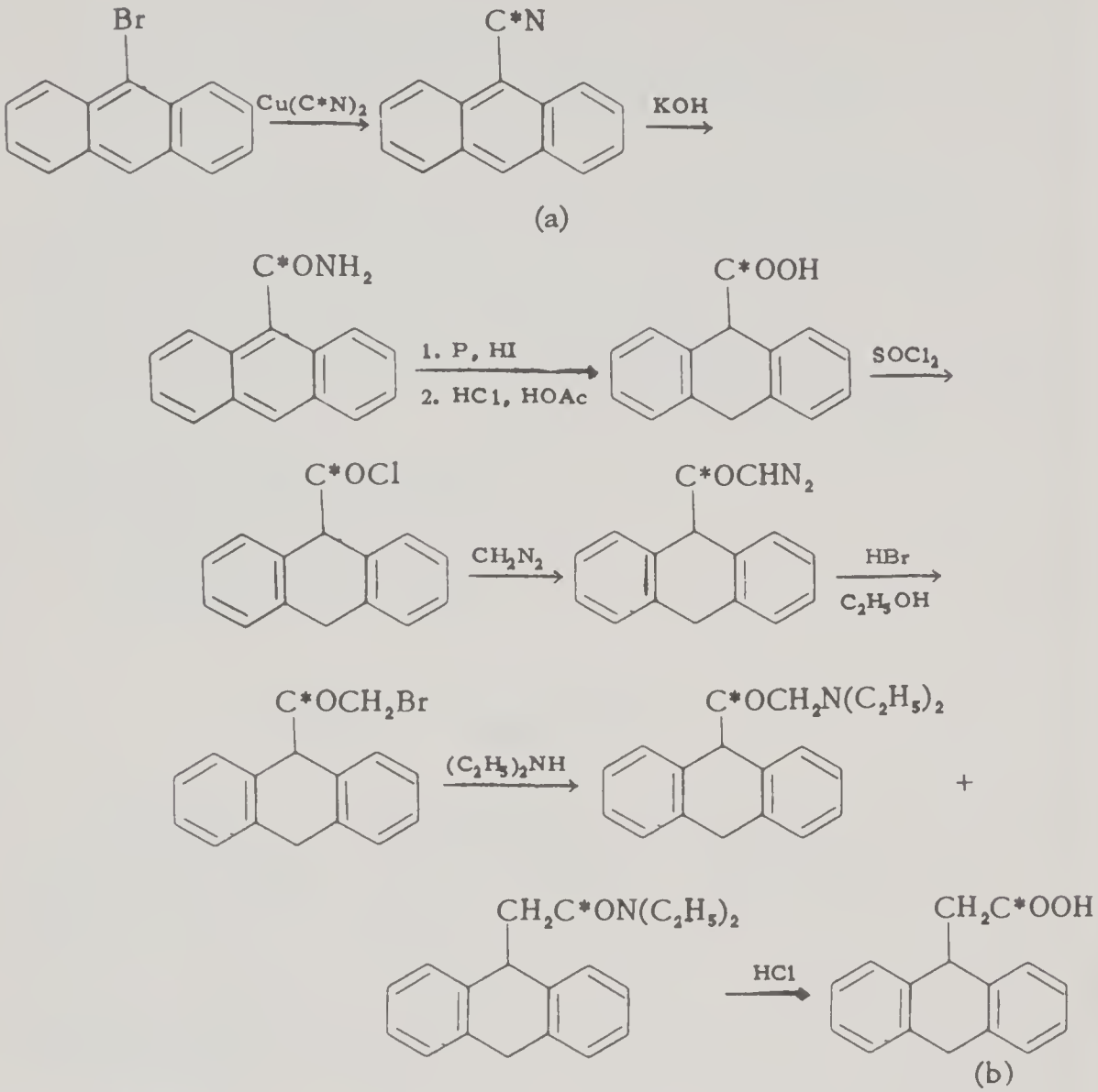
4. Anhydrous hydrogen bromide may be prepared by direct combination of the elements,[5,6] or by dropping liquid bromine into boiling tetrahydronaphthalene.[6,7]

5. The (halomethyl)naphthalenes are vessicants and may be polymerized by prolonged contact with acids. 1-(Bromomethyl)naphthalene melts at 56° and boils at 175° (10 mm.) (145–150° at 1 mm.).

6. 1-Naphthaleneacetonitrile (f.p. 5°) boils at 183–187° (13 mm.), 175–185° (11–14 mm.), 150–152° (1.5 mm.).

[1]R. F. Nystrom and W. G. Brown, J. Am. Chem. Soc., 69, 2548 (1947). *Organic Reactions*, Vol. VI, Wiley, New York, 1951, Chapter 10.
[2]A. Cambron, Can. J. Research, *17B*, 10 (1939).
[3]R. H. F. Manske and A. E. Ledingham, *ibid.*, *17B*, 14 (1939).
[4]H. de Pommereau, Compt. rend., *175*, 105 (1922).
[5]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 338.
[6]*Inorganic Syntheses*, Vol. I, McGraw, New York, 1939, p. 149.
[7]Houben, *Die Methoden der Organischen Chemie*, 3rd. ed., Vol. III, Verlag Gerog Thieme, Leipzig, 1930, p. 1156.

9,10-DIHYDRO-9-ANTHRACENEACETIC-C^{14} ACID



W. F. Dauben, C. F. Hiskey and M. A. Muhs, J. Am. Chem. Soc., 74, 2082 (1952).

A. Procedure (Note 1)

(a) *9-Anthronitrile-C^{14}*, (*9-Anthracenecarbonitrile-C^{14}*). The preparation is made from 12.8 g. (142 mmoles) of dry cuprous cyanide-C^{14} and 32.4 g. (127 mmoles) of 9-bromoanthracene[1] according to the procedure of Bachmann and Kloetzel,[2] which follows; yield 26.6 g. (92.4%), m.p. 172–173°.

To 2.0 ml. of dry pyridine is added 1.0 g. of dry cuprous cyanide, followed by 2.57 g. of 9-bromoanthracene, after the vigorous reaction subsides (Note 2). The mixture is heated at 220° for 9 hours, cooled, and shaken with dilute ammonium hydroxide and ether. The ether extract is washed with water, dilute hydrochloric acid and water, then dried and evaporated, and the residue is crystallized from alcohol; yield 1.76 g. (87%), m.p. 174–175°.

(b) *9,10-Dihydro-9-anthraceneacetic-C^{14} Acid.* 9-Anthronitrile-C^{14} is converted by the procedure of May and Mosettig,[3,4] which follows, methylene chloride being used as solvent in the preparation of the intermediate diazoketone. The over-all yield of acid is 12.4%, m.p. 171–172°; the molar specific activity is the same as that of the starting material.

9-Anthramide-C^{14}. A mixture of 18.8 g. of 9-anthronitrile, 17 g. of potassium hydroxide, 10 ml. of water and 75 ml. of ethylene glycol monoethyl ether is refluxed for 2.5 hours, then diluted with water. The product is collected (yield 20.5 g., m.p. 212–215°) and recrystallized from aqueous ethanol. m.p. 215–216°.

9,10-Dihydro-9-anthroic-C^{14} Acid (Note 3). A mixture of 20.5 g. of 9-anthramide, 5.6 g. of red phosphorus, 14 ml. of 57% hydriodic acid and 150 ml. of glacial acetic acid is refluxed for 7 hours. The hot filtered solution is diluted with 2 volumes of water, and the precipitate is collected, washed with water and refluxed for 2 hours with 60 ml. of concentrated hydrochloric and 120 ml. of glacial acetic acids. The solution is diluted with 450 ml. of water and cooled to 0°. The crude product is collected, dissolved in boiling aqueous sodium carbonate solution and reprecipitated by acidifying the filtrate; yield 17 g. (82%), m.p. 199–202°.

9,10-Dihydro-9-anthroyl-C^{14} Chloride. A mixture of 0.5 g. of 9,10-dihydro-9-anthroic acid and 1 ml. of purified thionyl chloride is refluxed for 1 hour and evaporated to dryness under vacuum (Note 4).

Diazomethyl 9,10-Dihydro-9-anthryl Ketone-C^{14} (Note 5). To a stirred, ice-cooled solution of diazomethane (from 2.5 g. of 1-nitroso-1-methylurea) in dry ether is added dropwise a solution of 1.7 g. of 9,10-dihydro-9-anthroyl chloride in 25 ml. of absolute ether. The mixture is stirred for 15 minutes at 0° and 2 hours at room temperature, then concentrated and diluted with ligroin; yield 1.2 g., m.p. 104–106.5° (dec).

Bromomethyl 9,10-Dihydro-9-anthryl Ketone-C^{14}. To 50 ml. of stirred, dry ether containing the diazomethyl ketone prepared from 3.5 g. of 9,10-dihydro-9-anthroyl chloride is added, dropwise at 15–20°, a solution of 3.5 ml. of 48% hydrobromic acid in 3.5 ml. of ether (Note 6). The mixture is stirred for 15 minutes at room temperature, then washed with dilute sodium carbonate, dried and concentrated to 10–15 ml. The solution is diluted with an equal volume of ligroin (b.p. 30–60°) and cooled; yield 3.1 g. of bromo ketone (73%), m.p. 104–105°. Recrystallization from ether-ligroin or methanol raises the melting point to 107–108°.

N,N-Diethyl-9,10-dihydro-9-anthraceneacetamide-C^{14} (Note 1). A mixture of 5.0 g. of bromomethyl ketone, 5 ml. of diethylamine and 40 ml. of absolute ether is stirred for 30 minutes (Note 7). The solution is diluted with 20 ml. of ligroin and cooled with an ice-bath. The precipitate is collected (4.4 g.) and digested with water, leaving 2.2 g. of product (45%), m.p. 133–135° (Note 8).

The mother liquor is washed with 3 portions of water, dried and acidified with hydrogen chloride, giving 1.5 g. of diethylaminomethyl 9,10-dihydro-9-anthryl ketone-C^{14} hydrochloride, m.p. 198–202° (Note 9).

9,10-Dihydro-9-anthraceneacetic-C^{14} Acid. A mixture of 1.0 g. of the amide, 8 ml. of concentrated hydrochloric acid and 2 ml. of glacial acetic acid is refluxed for 1 week, during which time 4 ml. of hydrochloric and 1 ml. of acetic acids are added. The mixture is diluted with water and cooled, and the precipitate is collected. The product is separated from 0.15 g. of starting material by dissolution in boiling dilute sodium carbonate solution. Acidification of the filtrate gives 0.6 g. of the acid (90% yield based on unrecovered amide), m.p. 165–168°. Recrystallization from methanol-water raises the melting point to 168–169.5° (Note 10). Degradation studies (Note 11) indicate that all the isotope is located in the carboxyl group. The molar specific activity is the same as that of the starting materials.

B. Notes

1. The preparations are incidental to a study of the reaction of α-haloketones with bases; see isopentyl cyclopentanecarboxylate-$C^{14}_{1/1}$-1,3-$C^{14}_{1/2}$—the Faworskii rearrangement[3,5]. The ease of removal of the α′-hydrogen greatly influences the course of reaction; in the present instance the activation of the α′-hydrogen by two phenyl groups apparently allows the reaction to proceed in the presence of such weak bases as secondary amines, forming the rearrangement product (40–50% yield) and the displacement product (30–40% yield). The similar benzhydryl halomethyl ketones, however, rearrange only in the presence of the stronger sodium methoxide.

2. The system is protected from moisture by a mercury trap.

3. Phosphorus-hydriodic acid reduction provides an excellent means of converting 9-substituted anthracenes to the corresponding 9,10-dihydroanthracene derivatives.[4]

4. Benzene may be used as solvent in this reaction,[4] and the product may be recrystallized from ligroin (b.p. 30–60°), giving prisms (90%), m.p. 57–58°.

5. The Arndt-Eistert synthesis is discussed by Bachmann and Struve.[6]

6. There is a vigorous evolution of gas.

7. Cooling is necessary at first.

8. Recrystallization from methanol raises the melting point to 134–135°.

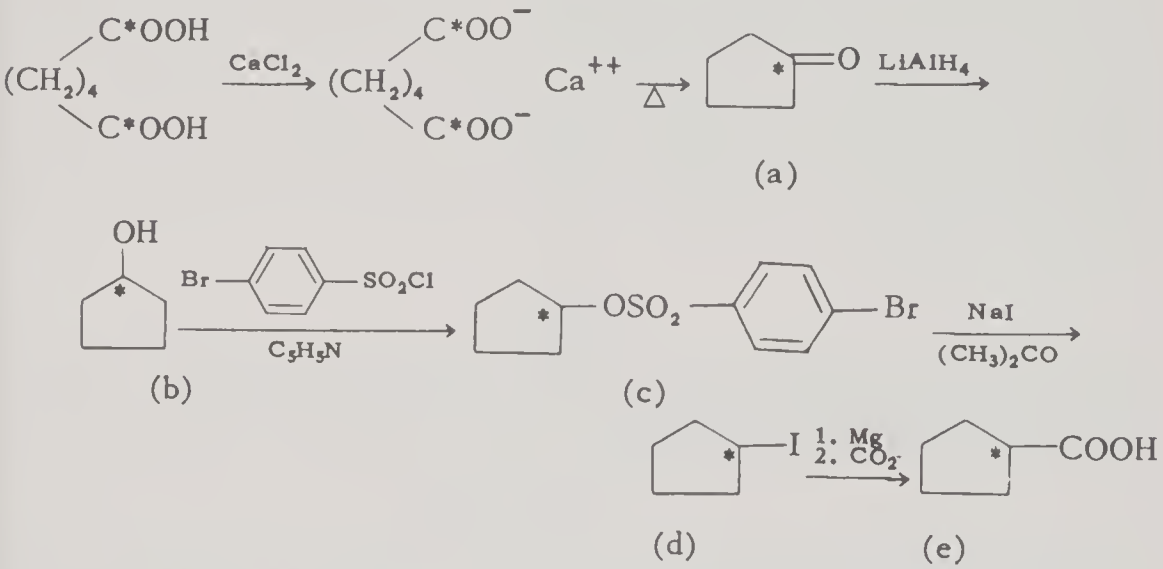
9. The picrate melts at 127–129°.

10. The acid appears to be oxidized slowly in air.

11. Directions are given by Dauben for decarboxylating and dehydrogenating the acid with copper chromite, forming nonisotopic 9-methylanthracene, m.p. 75–77°.

[1]E. de B. Barnett and J. W. Cook, J. Chem. Soc., *125*, 1084 (1924).
[2]W. E. Bachmann and M. C. Kloetzel, J. Org. Chem., *3*, 55 (1938).
[3]E. L. May and E. Mosettig, J. Am. Chem. Soc., *70*, 1077 (1948).
[4]E. L. May and E. Mosettig, J. Am. Chem. Soc., *70*, 688 (1948).
[5]R. Jacquier, Bull. soc. chim. France, D35 (1950); G. Richard, Compt. rend., *197*, 1432 (1933); W. D. McPhee and E. Klingsberg, J. Am. Chem. Soc., 66, 1132 (1944).
[6]*Organic Reactions*, Vol. I, Wiley, New York, 1942, p. 38.

CYCLOPENTANECARBOXYLIC-1-C^{14} ACID



R. B. Loftfield, J. Am. Chem. Soc., 73, 4707 (1951).

A. Procedure

(a) *Cyclopentanone-1-C^{14}*. This next lower cyclic ketone is prepared from hexanedioic-1,6-C_2^{14} acid by the same procedure used in preparing cyclohexanone-1-C^{14} (q.v.) from heptanedioic-1,7-C_2^{14} acid.

(b) *Cyclopentanol-1-C^{14}*. A solution of 13.0 g. of cyclopentanone-1-C^{14} in 50 ml. of absolute ether is added dropwise to a stirred solution of 4.0 g. of lithium aluminum hydride in 100 ml. of ether. The excess hydride is decomposed with dilute hydrochloric acid. The yield of distilled product is 11.2 g., b.p. 54–55° (22 mm.).

(c) *Cyclopentyl-1-C^{14} p-Bromobenzenesulfonate*. To a solution of 7.8 g. of cyclopentanol-1-C^{14} in 50 ml. of dry pyridine at 0° is added 25 g. of *p*-bromobenzenesulfonyl chloride. The mixture is stored overnight in an ice-bath, then poured into excess cold, dilute hydrochloric acid, and the ester is extracted into carbon tetrachloride. The extract is dried over potassium carbonate and evaporated to dryness. The yield, after two recrystallizations from petroleum ether, is 20.4 g., m.p. 45.5–46.0° (Note 1).

(d) *1-Iodocyclopentane-1-C^{14}.* A mixture of the ester, 60 g. of acetone and 15 g. of sodium iodide is stored overnight under nitrogen. The salt is filtered off and washed with petroleum ether. The combined filtrate is poured into 200 g. of ice-water containing 1.0 g. of sodium bisulfite, and the product is extracted into two portions of petroleum ether, dried and distilled; yield 9.8 g., b.p. 57–58° (17 mm.).

(e) *Cyclopentanecarboxylic-1-C^{14} Acid.* A solution of 9.8 g. of the iodide in 100 ml. of absolute ether is added over 4 hours to a stirred mixture of 1.5 g. of magnesium turnings and 50 ml. of ether. The Grignard reagent is carbonated at −5° by passing carbon dioxide through the stirred mixture. The complex is hydrolyzed with dilute sulfuric acid, and the ether layer is separated, washed with water and extracted with sodium hydroxide solution. The alkaline extract is washed with ether, then acidified. The yield of product, obtained by distillation, is 2.0 g., b.p. 111–112° (17 mm.). Degradation studies indicate that the isotope occurs only at C-1 (Note 2).

B. Notes

1. The ester decomposes in a few days at room temperature.
2. See cyclopentanone-1,2-$C^{14}_{1/2}$.

C. Other Preparations

(C^{14}-Cyclopentane)carboxylic-1-$C^{14}_{1/1}$ acid has been prepared by Loftfield,[1] by the sequence: cyclopentanol-1-C^{14}, C^{14}-1-bromocyclopentane (*via* PBr_3), (C^{14}-cyclopentane)carbonitrile-1-$C^{14}_{1/1}$ and (C^{14}-cyclopentane)-carboxylic-1-$C^{14}_{1/1}$ acid. However, degradation studies showed that rearrangement had taken place, probably in the first step, as 20% of the activity was found in the degradation product C^{14}-putrescine (Note 2).

[1]R. B. Loftfield, J. Am. Chem. Soc., *73*, 4707 (1951).

BENZOIC-C^{14} ACID

$$RMgX \xrightarrow{C^*O_2} RC^*OOMgX \xrightarrow{H^+} RC^*OOH$$

W. G. Dauben, J. C. Reid and P. E. Yankwich, Anal. Chem., *19*, 828 (1947).
C. Heidelberger and H. S. Rieke, Cancer Research, *11*, 640 (1951).

A. Procedure (Note 1)

The apparatus consists of a high-vacuum manifold equipped with attachments to a reaction flask, carbon dioxide generator, mercury manometer and a nitrogen inlet and outlet; see Figure II, 5. The cone-shaped,

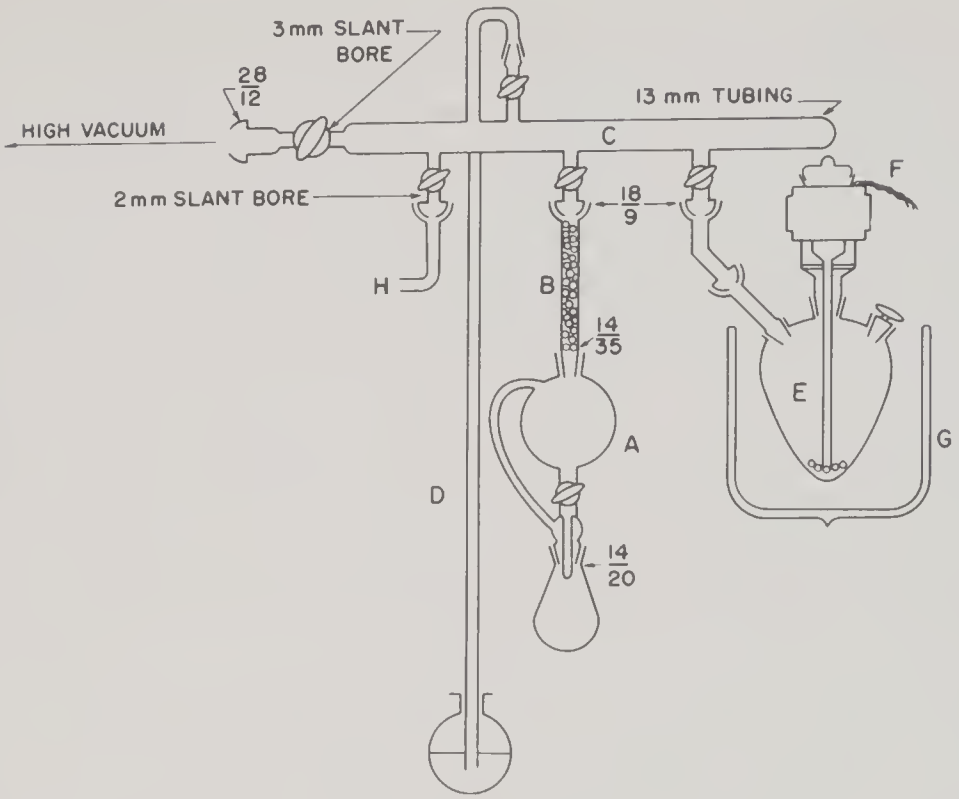


Fig. II, 5. Grignard carbonation apparatus (W. G. Dauben, J. C. Reid and P. E. Yankwich). A, carbon dioxide generator; B, Drierite; C, vacuum manifold; D, mercury manometer; E, reaction flask; F, induction stirrer (110 volt, 3 phase); G, cooling bath; H, nitrogen inlet.

three-necked reaction flask, designed to allow freezing, is fitted with an overhead induction stirrer for use under vacuum with cooling baths (Note 2). The generator consists of a round-bottomed flask, containing barium carbonate-C^{14}, and which is fitted with a pressure-equalizing addition funnel containing concentrated sulfuric acid (Note 3) and attachment to a drying tube (Note 4).

The Grignard reagent is prepared under dry nitrogen (Note 5) in an all-glass apparatus, and an aliquot of the ethereal solution is assayed by titration (Note 6). The system is evacuated to 0.1μ and filled with dry nitrogen. The carbonation flask is charged quickly with a 10% excess of the Grignard reagent (Note 7), by means of a pipette previously flushed with nitrogen and operated by a syringe. The side arm stopper is replaced, the solution is frozen with liquid nitrogen, and the system is evacuated to 0.1μ (Note 8). The mixture is thawed with a Dry Ice-acetone bath at -20° and refrozen with liquid nitrogen; then the system is evacuated to remove entrapped nitrogen. The stirred Grignard reagent then is carbonated at -20° (Note 9) by dropping sulfuric acid slowly onto the barium carbonate, while the pressure is kept below 50 cm. The last of the gas is evolved by warming the generator gently to dissolve the salts. Absorption is complete when the manometer reading is constant, 5–15 minutes being required for the process. The mixture is frozen with

liquid nitrogen to draw any remaining carbon-C^{14} dioxide into the reaction flask, the stopcock to the generator is closed, and the mixture is stirred for 15 minutes at -20° to ensure complete absorption (Note 10). The system is filled with nitrogen and vented to the atmosphere; then the complex is decomposed with dilute hydrochloric or sulfuric acid. The acid mixture is extracted with ether, and the product is extracted from the ether solution with dilute sodium hydroxide (Note 11). The alkaline solution is acidified, and the product is collected by filtration, ether extraction or steam distillation; yield 85–95% or more (Note 12).

Benzoic-C^{14} Acid. A Grignard solution is prepared from 0.6 g. of magnesium and 3.9 g. of bromobenzene in absolute ether. Phenylmagnesium bromide (12.8 mmoles, 20 ml., 0.63 *M*) is carbonated with the carbon-C^{14} dioxide liberated from 1.953 g. (10.0 mmoles) of barium carbonate-C^{14}. Following hydrolysis, the ethereal solution is extracted exhaustively with dilute sodium hydroxide; then the alkaline solution is washed with ether, acidified and extracted with ether, which in turn is extracted with a small volume of sodium hydroxide solution. The hot aqueous solution is acidified and cooled to precipitate the product; yield 1.036 g. The mother liquor is extracted with ether, and the ethereal solution is extracted with alkali and acidified, yielding an additional crop, making the total yield 1.139 g. (94%), m.p. 119–121° (Note 13).

B. Notes

1. The procedure is general for the carbonation of Grignard reagents with carbon-C* dioxide. The method is a modification of those described by Sakami,[1] Buchanan,[2] Spector,[3] Van Bruggen[4] and Leslie.[5]

2. Several diagrams of the apparatus are presented by Dauben. Diagrams of a number of vacuum-tight magnetically-stirred reaction flasks have been published.[6]

3. It is advantageous to employ previously out-gassed sulfuric acid, a glass-enclosed magnetic stirring bar and a plug of glass wool to prevent blowing barium carbonate dust into the drying train. Any dust spattered onto the walls may be reached with acid by rotating the generator about its median semi-ball joint connection to the drying trap.[6c] A volume of 5–8 ml. of acid is used per gram of carbonate, and the stopcock is lubricated with Silicone grease

4. Carbon-C^{14} dioxide may be purified by slow passage through a spiral trap cooled with Dry Ice. The vapor pressure of water at -78° is 0.6μ, while over 95% sulfuric acid at 25° it is considerably less than 1μ.

5. Oxygenation of a Grignard reagent introduces an error into the determination of the concentration by simple titration and gives rise to carbinols,[7] which are difficult to remove in the case of arylmagnesium halides, due to their phenolic (acidic) nature. Moisture, of course, reduces the concentration of reagent.

6. The sample is pipetted into an excess of 0.1 *N* hydrochloric acid, and the excess is determined by titration with sodium hydroxide in the presence of methyl orange indicator.[8] Gilman,[9] in a study of the yields of some Grignard reagents, has developed an improved titration procedure: an aliquot is heated with an excess of standard sulfuric acid, then titrated with alkali to the end-point of phenolphthalein.

7. The concentration of Grignard reagent is kept usually at, or below, 0.25 *M* to minimize ketone and carbinol formation and to keep the reagent in solution at the temperature of carbonation. A large excess of reagent serves no useful purpose.

8. The stopcock to the vacuum-manifold is opened momentarily to remove an appreciable amount of the nitrogen before the solution solidifies. The absorption of carbon dioxide is facilitated when the inert gas is removed, the manometer then registering the same pressure before and after carbonation.

9. Sometimes the stirred Grignard reagent is cooled with a Dry Ice-bath and allowed to warm to room temperature in contact with carbon-C^{14} dioxide. The lowered vapor pressure of ether, at low temperature, permits faster diffusion of the carbon dioxide into the mixture.

10. When high specific activity carbonate is used, it is advisable to recover any unreacted carbon-C^{14} dioxide by distillation at -20° into a trap cooled with liquid nitrogen.

11. The by-products, arising from side reactions (R_2C^*O and R_3C^*OH), coupling (R-R) and hydrolysis (RH), are readily separated from the product by virtue of their nonacidic character.

12. The conditions minimizing the formation of ketone and carbinol are: low concentration, a small excess of the Grignard reagent, short carbonation time, effective mixing and low reaction temperature. It is difficult to evaluate the relative importance of these factors, some of which may not be critical in certain cases.

13. Dauben has reported a yield of 85.4%, m.p. 122–123°, at the 29-mmole scale, without working up the mother liquor.

14. A diagram of the simplified hand-shaken apparatus and detailed directions for its operation are presented. The carbonation is effected at -10°, and the product is purified by steam distillation from an ether extract after addition of dilute sulfuric acid and silver sulfate. The sodium salt is formed by titration.

15. Contrary to the findings of Spector,[3] an excess of Grignard reagent proved to be favorable.

16. A systematic study was made of the effect of temperature, concentration and mole proportions upon yield.

17. The absorption of carbon dioxide was 80–90% during 20 minutes at -20°. The yield is based on reacted carbon dioxide.

18. The carbonation temperature was −30°, and the product was isolated from the steam distillate as the silver salt.

19. The yield is on a radiochemical basis, achieved with the aid of 50 g. of carrier: 44 g. of acetic-1-C^{14} acid (88%), b.p. 102–102.3° (490 mm.), and 22 g. of sodium acetate-1-C^{14} (10%).

20. The carbonation was run overnight at −15°. The recovery of unreacted carbon dioxide was 5%.

21. The procedure is based on 50 trial reactions at the sub-millimole scale with a small, compact apparatus not requiring a high-vacuum manifold. Macdonald[10] has reported obtaining a yield of only 50% in an isotopic preparation by this procedure.

22. Carbonation was effected by shaking at room temperature for 10 minutes.

23. Carbonation was effected by bubbling a stream of nitrogen containing the carbon dioxide through the Grignard mixture at 0°.

24. The procedure was that described by Van Bruggen.[4] The sodium acetate-1-C^{14} was converted to the free acid and concentrated by distillation and extraction procedures. The over-all yield of 10 *N* acid was 60%. A number of diagrams of semimicroapparatus are presented, Figure II, 6. A more effective method for preparing the free acid from the salt appears to be with anhydrous hydrogen chloride and the use of a vacuum line; see sodium acetate-1-C^{14} Note 6.

25. The preparation was by the method of Sakami.[1]

26. The carbonation was performed at 0° to −25° at a pressure above 200 mm., and the complex hydrolyzed—all within 5 minutes.
The product was concentrated by distillation at 115 mm., followed by continuous ether extraction. The redistilled product was about 83% pure by weight. The effects of time, temperature and concentration were

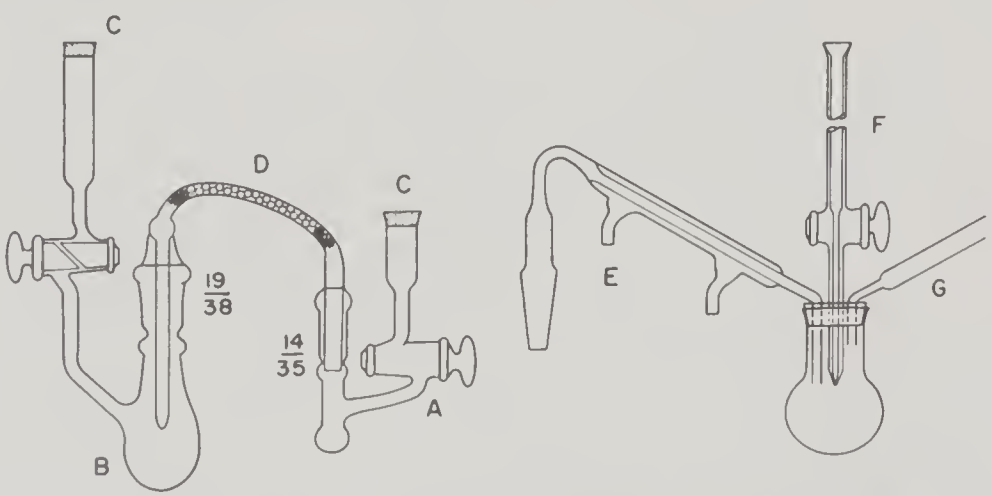


Fig. II, 6. Grignard carbonation and acid distillation apparatus (J. T. Van Bruggen, C. K. Claycomb and T. T. Hutchens). A, carbon dioxide generator (5-ml. reservoir): B, carbonation and distillation flask; C, rubber vial closure: D, Anhydrone: E, distillation assembly; F, tip of a microburette; G, soda-lime.

studied; a 100% excess of 0.35 *M* Grignard solution was best. A diagram of the hand-shaken apparatus is given.

27. Carbonation was achieved by shaking for 10 minutes at 0°, and the product was isolated by extraction.

28. Carbonation was achieved by shaking at room temperature for a few minutes. Silver sulfate was added to prevent distillation of hydrobromic acid with the product.

29. The acid is isolated as the barium salt by steam distillation under vacuum at 50–60° in the presence of silver sulfate-sulfuric acid, and titration with barium hydroxide and evaporation. A diagram of the vacuum system is given by Neyman.[11] Yields of 95–97% were obtained in trial preparations by adding a cooled 0.5 *N* ethereal Grignard solution to solid carbon dioxide in a cooled reaction vessel, then decomposing the complex with 18 *N* sulfuric acid.

30. Directions are given for preparing an anhydrous product of low specific activity. The Grignard complex was decomposed with a solution of hydrogen chloride in ether; then 1 g. of carrier propionic anhydride and 10 g. of carrier propionic acid were added. The mixture was vacuum-distilled and chased with two 10-g. portions of carrier propionic acid. Fractionation of the combined distillates gave 23.5 g. (64%) of propionic-1-C^{14} acid, b.p. 100.5–101.5° (205 mm.). An additional 23% was recovered as the sodium salt by acidification and steam distillation of the Grignard residue.

31. B.p. 184–189°.

32. The preparation was according to the procedure of Leslie.[5]

33. The carbonation mixture was chilled overnight before being decomposed. The yield of vacuum-distilled product is based on the halide.

34. The product is purified by vacuum distillation. The *p*-bromophenacyl ester melts at 54° (cor.).

35. The carbonation was carried out at −10°. The product melted at 33.5°.

36. Directions are outlined for preparing 1-chloro-4-methoxybutane from 1,4-butanediol by the action of methyl iodide on the monosodium derivative, followed by treatment with phosphoros trichloride; b.p. 142–144°, n_D^{16} 1.4272.

37. Cyclopentanecarboxylic-C^{14} acid was reduced by cold ethereal lithium aluminum hydride solution, forming cyclopentanemethanol-α-C^{14} in nearly quantitative over-all yield.

38. The product was distilled at 200–202°, n_D^{26} 1.4143.

39. 3-Bromoheptane is prepared by the method of Sherill.[12] The yield is based on unrecovered carbon-C^{14} dioxide.

40. No perceptible volatile acidic impurity was detected by a Duclaux distillation. The neutralization equivalent was 143.0 (calc. 144.2). The

isotopic content was in agreement that value calculated from the starting material.

41. The purified product melted at 39.5–41.5°, n_D^{60} 1.427, and the molar specific activity agreed with that of the starting material.

42. The acid was treated with thionyl chloride, and the resulting stearoyl-C^{14} chloride was converted to *N,N*-dibutylstearamide-C^{14} by reaction with dibutylamine. Methyl-C^{14} stearate has been prepared in a similar manner[13] by treating the acid chloride with methanol-C^{14}, m.p. 37.8–38° after recrystallization from methanol.

Directions are given by Bergström[14] for preparing the isotopic glyceride by transesterification with triolein.

43. The product distilling at 70–105° (60 mm.) was collected.

44. The yield is based on the halide.

45. M.p. 94.5–95.5°.

46. M.p. 114–115°.

47. M.p. 113–114°.

48. M.p. 127–129°. The low yield was attributed to insufficient purification of the starting material.

49. M.p. 148.5–149°.

50. M.p. 83–84°.

51. The yield was 42% after recrystallization from benzene, m.p. 148°.

52. The frozen mixture of carbon dioxide and Grignard reagent was thawed with a bath at –15°, and the complex was decomposed 10 hours later. The product from a trial preparation distilled at 127° (0.1 mm.)

53. A higher yield was obtained by carbonating at –40° to –45° than at –20°. The yield is based on reacted carbon dioxide, 13.3 mmoles being recovered. Recrystallization from petroleum ether raised the melting point to 57.5–59°. Trial experiments with 3-phenylpropyl bromide gave 4-phenylbutyric acid in 90–96% yield.

54. The yield was 90–91% at the 13- to 14-mmole scale.

55. The carbonation method was that of Van Bruggen;[4] radiochemical yield 81%, m.p. 250° (sealed tube).

56. A diagram of the apparatus and detailed instructions are presented by Barker.[15]

57. M.p. 99.8–100.8°.

58. M.p. 151–153° from benzene.

59. The procedure was adapted from the method of Barnes.[16] Stephens[17] has determined the relative isotope effects of C^{13} and C^{14} during decarboxylation.

60. The sluggish preparation of the Grignard reagent was completed by refluxing for 6 hours in 50% benzene solution. The sparingly soluble reagent was dissolved by dilution with ether-benzene.

61. *p*-Anisic-C^{14} acid was demethylated to *p*-hydroxybenzoic-C^{14} acid by treatment with aluminum bromide in benzene by the method of Pfeiffer.[18]

62. The carboxylation is carried out at the temperature of an ice-salt-bath, 10% of the carbon-C^{14} dioxide being recovered. The product is precipitated from 1 ml. of alkaline solution with 6*N* hydrochloric acid: m.p. 98–99°.

63. *p*-Bromophenyl isopropyl ether, m.p. 17.5° from light petroleum, is converted to the Grignard reagent as in Note 65. The acid is crystallized from alcohol, m.p. 159° (softens at 140°).

64. The α-phenyl-*o*-toluic-C^{14} acid is converted to *o*-benzoylbenzoic-C^{14} acid, n.p. 127°, by oxidation with potassium dichromate in acetic acid.

65. *p*-Bromophenyl cyclohexyl ether,[20] b.p. 115–117° (0.8 mm.) is converted to the Grignard reagent by boiling with magnesium under nitrogen for 5 hours in the presence of a few drops of ethyl iodide; the filtered solution is carbonated. The acid crystallizes from alcohol, m.p. 176°.

66. The yield was 80–85% based on unrecovered carbon dioxide.

67. The carbonation was run in an ether-benzene mixture at 0°. The product melted at 104–107°.

68. Carbonation was effected at −70°, and the Grignard complex was stored overnight at room temperature. After two reprecipitations with acid from alkaline solution, the product melted at 114°.

69. The carbonation was carried out at −10°. The product melted at 111–112°.

70. The starting material is prepared from 3-(ethylthio)-1-propanol and phosphorus tribromide in carbon tetrachloride. The product is distilled under high vacuum at 100°, n_D^{22}° 1.4805. Paper chromatography and radioautography demonstrate the presence of a trace impurity of R_f 0.12: see (ethylthio)acetic-1-C^{14} acid, Note 3.

71. The acid was reduced with excess lithium aluminum hydride in ether solution, and the 1-undecanol-1-C^{14} was converted to 1-iodo-undecane-1-C^{14} with 60% hydriodic acid by the method of Guyer.[21] Further reduction with lithium aluminum hydride gave undecane-1-C^{14}; radiochemical yield 44.8%.[76]

72. The carbon-C^{14} dioxide generated by the use of 72% perchloric acid is swept by a continuous stream of nitrogen into the stirred Grignard solution (448 mmoles) at −55° during 5–6 hours. The product melts at 76.5–78.0°.

73. The carbonation was accomplished during 15 minutes at room temperature; a diagram of a simple hand-shaken apparatus is presented. The acid was converted to 3-methylbutyryl-1-C^{14} chloride by the action of thionyl chloride. Directions are given for preparing *p*-bromophenacyl isovalerate-C^{14} on a microscale by refluxing an aqueous alcohol solution containing the sodium salt, 2,4'-dibromoacetophenone and a drop of very

dilute hydrochloric acid. A microrecrystallization apparatus is described by Hallett.[18a]

74. A mixture of 0.3 g. of magnesium chips, a crystal of iodine and a solution of 2.55 g. of 2-benzyloxy-5-bromoanisole[106] in 35 ml. of absolute tetrahydrofuran (ether is unsatisfactory) under nitrogen is slowly brought to the boiling point and refluxed for 2 hours. The acid obtained by carbonation is converted to vanillic-C^{14} acid by hydrogenolysis.

75. Isomerization in hot 50% sulfuric acid[37] gave crotonic-1-C^{14} acid; yield 4.9%, m.p. 70.5–72° from petroleum ether. Also see sodium acetate-2-C^{14}, Table II, 2.

76. Alternate layers of Grignard reagent and carbon-C^{14} dioxide are frozen out in an apparatus of the type described by Van Bruggen;[4] a diagram is given by Logan.[22a] The carbonation is completed at 0°.

77. An apparatus diagram of the Sakami[1] type and directions are given by Pearce.[109] The Grignard reagent is added to solid carbon-C^{14} dioxide.

78. The carbonation was run at 0°; the acid melts at 162.5–163.5°.

79. The carbonation temperature was –60°; the product melts at 103–105°.

C. Other Preparations

By similar procedures, benzoic-C^{14} acid has been prepared: Stevens[19] (80–85%), Bills[22] (91.6%), Geissman[23] (92–95%), Jerchel[24] (80%), Schmid[25] (92%), Bergel[26] (89%), Pines[27] (83% at the 30.2-mmole level), Bernhard[28] and Koton[29] (73–76%); and the –C^{13} acid by Huggett.[30] Baret[67] has obtained yields of 78–88%, after a study of the effects of temperature, concentration and molecular proportions upon yield.

Many carboxylic-C* acids are prepared by this general method; see Table II, 3.

[1]W. Sakami, W. E. Evans and S. Gurin, J. Am. Chem. Soc., *69*, 1110 (1947).

[2]J. M. Buchanan, A. B. Hastings and F. B. Nesbett, J. Biol. Chem., *150*, 413 (1943).

[3]L. B. Spector, Atomic Energy Commission Report, MDDC-532; also see reference 36, p. 177.

[4]J. T. Van Bruggen, C. K. Claycomb and T. T. Hutchens, Nucleonics, 7 (3), 45 (1950).

[5]W. B. Leslie, Atomic Energy Commission Report, MDDC-674.

[6a]W. G. Dauben, J. C. Reid and P. E. Yankwich, Anal. Chem., *19*, 828 (1947).

[6b]B. M. Tolbert, J. Biol. Chem., *173*, 205 (1948).

[6c]A. Murray III, W. W. Foreman and W. Laugham, J. Am. Chem. Soc., *70*, 1037 (1948).

[6d]C. J. Collins, J. Am. Chem. Soc., *70*, 2418 (1948).

[6e]B. M. Tolbert, W. G. Dauben and J. C. Reid, Anal. Chem., *21*, 1014 (1949).

[6f]B. M. Tolbert, F. Christenson, F. N.-H. Chang and P. P. T. Sah, J. Org. Chem., *14*, 525 (1949).

TABLE II, 3.

Carboxy-C* Acid Synthesis by Grignard Carbonation

Acid	RX	mmole	% yield	Ref.	Note
Acetic	CH_3I	20	95–98	30a,31	–
Acetic	CH_3I	5	100	32	14
Acetic	CH_3I	0.1–15	92–97	33	15,16
Acetic	CH_3I	50	92–96	34	–
Acetic	CH_3I	–	up to 96	35	17
Acetic	CH_3I	18	89–94	36	–
Acetic	CH_3I	24	91	37	18
Acetic	CH_3Cl	52	98	38	19
Acetic	CH_3Cl	20.3	89	39	–
Acetic	CH_3I	50	89	12	20
Acetic	CH_3I	25	85	40	–
Acetic	CH_3I	0.25–1.0	80–90	11	21
Acetic	CH_3I	10	74	10	21
Acetic	CH_3I	1	95	41	22
Acetic	CH_3I	50	91	109	–
Acetic	CH_3I	125	89	42	–
Acetic	CH_3I	10	92	43	–
Acetic	CH_3I	–	68–90	44	–
Acetic	CH_3I	0.7	69–77	45	–
Acetic	CH_3I	25	65–85	46	23
Acetic	CH_3I	1	75	47	24
Acetic	CH_3I	50	73	48	25
Acetic	CH_3I	100–185	70–87.5	9	26
Acetic	CH_3I ($C^{11}O_2$)	2	50	49	27
Propionic	CH_3CH_2I	–	95–98	30	–
Propionic	CH_3CH_2I	20	97.8	50	–
Propionic	CH_3CH_2I	46	95	51	–
Propionic	CH_3CH_2I	–	96	52	–
Propionic	CH_3CH_2I ($C^{11}O_2$)	2	–	49	–
Propionic	CH_3CH_2Br	–	80	53	–
Propionic	CH_3CH_2Br ($C^{11}O_2$)	0.5	95	54	28
Propionic	CH_3CH_2Br	21	95	55	–
Propionic	CH_3CH_2Br	–	88	56	29
Propionic	CH_3CH_2Br ($C^{11}O_2$)	1.58	90	57	28
Propionic	CH_3CH_2Cl	52	87	38	30
Butyric	$CH_3CH_2CH_2X$	20	95–98	31	–
Butyric	$CH_3CH_2CH_2Br$ ($C^{13}O_2$)	–	–	58	–
Butyric	$CH_3CH_2CH_2X$ ($C^{11}O_2$)	2	–	49	–
Butyric	$CH_3CH_2CH_2Br$	–	95	59	–
3-Butenoic	$CH_2{=}CHCH_2Br$	–	–	107	75
3-Butenoic	$CH_2{=}CHCH_2Cl$	–	28	80	43
Isobutyric	$(CH_3)_2CHX$	20	95–98	31	–
Isobutyric	$(CH_3)_2CHBr$	20	97.5	60	–
Isobutyric	$(CH_3)_2CHBr$	51	59	61	–
Isobutyric	$(CH_3)_2CHBr$	–	–	62	–
Valeric	$CH_3(CH_2)_3X$	20	95–98	31	–
Valeric	$CH_3(CH_2)_3I$	–	70	56	31

(*continued*)

TABLE II, 3. (*continued*)

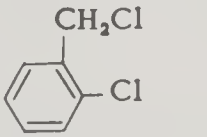
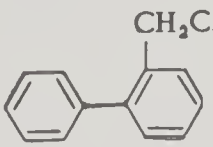
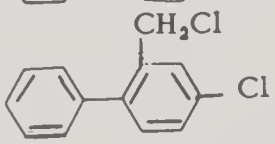
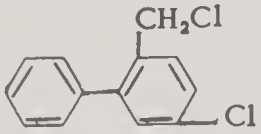
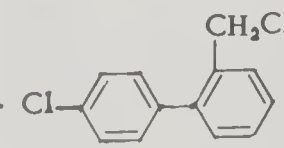
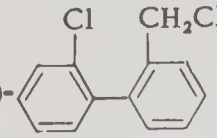
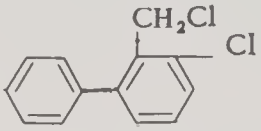
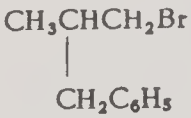
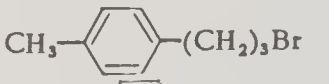
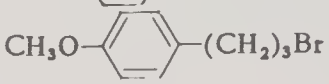
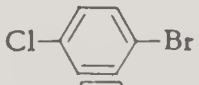
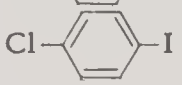
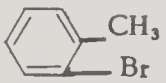
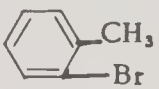
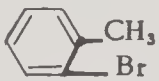
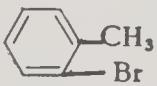
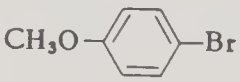
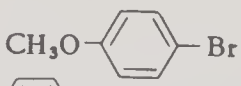
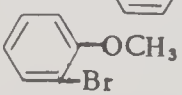
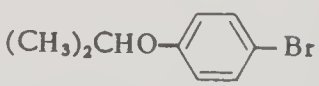
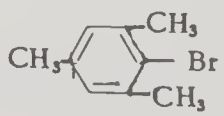
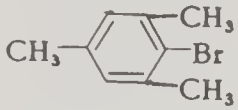
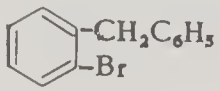
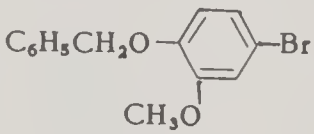
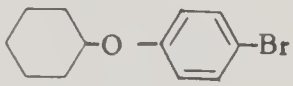
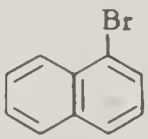
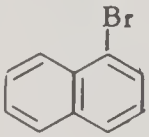
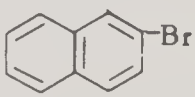
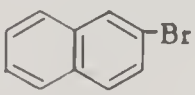
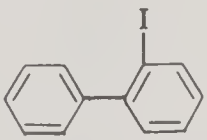
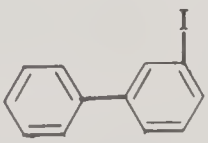
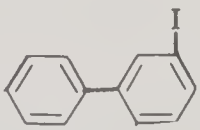
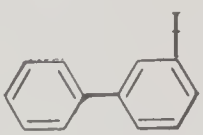
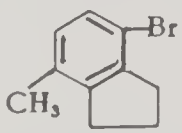
Acid	RX	mmole	% yield	Ref.	Note
Isovaleric	$(CH_3)_2CHCH_2X$	(20)	96	63	–
Isovaleric	$(CH_3)_2CHCH_2X$	(20)	95–98	31	–
Isovaleric	$(CH_3)_2CHCH_2Br$	1	83.6	14	73
Isovaleric	$(CH_3)_2CHCH_2Br$	3	89	64	32
Isovaleric	$(CH_3)_2CHCH_2Br$	54	65	65	33
2-Methylbutyric	$CH_3CH_2CHBrCH_3$	50.7	60	66	34
Trimethylacetic, (Pivalic)	$(CH_3)_3CCl$	0.1–1.5	74–79.5	33	16,35
5-Methoxyvaleric	$CH_3O(CH_2)_4Cl$	–	85–90	67	36
Cyclopentanecarboxylic	C_5H_9Br	–	100	68	37
Hexanoic, (Caproic)	$CH_3(CH_2)_4Br$	83.5	85.5	69	38
Hexanoic, (Caproic)	$CH_3(CH_2)_4Br$	–	71	70	–
4-Methylvaleric, (Isocaproic)	$(CH_3)_2CH(CH_2)_2Br$	(20)	95	63	–
Heptanoic	$CH_3(CH_2)_5Br$	–	–	71	–
2-Ethylhexanoic, (2-Ethylcaproic)	$CH_3(CH_2)_3CHBrCH_2CH_3$	17	80	72	39
Octanoic	$CH_3(CH_2)_6Br$	10	81	73	40
Octanoic	$CH_3(CH_2)_6Br$	–	72	74	–
Nonanoic	$CH_3(CH_2)_7Br$	–	–	71	–
Decanoic, (*n*-Capric)	$CH_3(CH_2)_8Br$	–	–	75	–
Undecanoic, (*n*-undecoic, hendecanoic, undecylic)	$CH_3(CH_2)_9Br$	0.19	–	76	71
Tridecanoic	$CH_3(CH_2)_{11}Br$	24	–	77	41
Pentadecanoic	$CH_3(CH_2)_{13}Br$	–	–	78	–
Stearic	$CH_3(CH_2)_{16}Br$	–	–	79,16	42
Cyclohexanecarboxylic	$C_6H_{11}Cl$	0.1–1.5	95–98	33	16,35
Phenylacetic	$C_6H_5CH_2Cl$	300	93.4	104	72
(*o*-Chlorophenyl)acetic		–	68.7	81	44,45
2-Biphenylacetic		49.2	69	82	44,46
(4-Chloro-2-biphenylyl)-acetic		42.2	75	82	44,47
(5-Chloro-2-biphenylyl)-acetic		34.7	32	82	44,48
(4′-Chloro-2-biphenylyl)-acetic		42.2	68	82	44,49

TABLE II, 3. (*continued*)

Acid	RX	mmole	% yield	Ref.	Note
(2′-Chloro-2-biphenylyl)-acetic		–	58	82	44,50
(3-Chloro-2-biphenylyl)-acetic		–	79	81	44,51
3-Methyl-4-phenylbutyric		5	91.5	83	52
4-*p*-Tolylbutyric		30	85.5	84	53
4-(*p*-Methoxyphenyl)-butyric		2.65	95	85	54
4-Phenylbutyric		–	65	86	–
p-Chlorobenzoic		1.34	83	87	55
p-Chlorobenzoic		1	78	22a	76
o-Toluic		10	–	88	–
o-Toluic		–	62	89	–
o-Toluic		10	85	17	56
o-Toluic		4.9	75	90	57
p-Anisic		5.2	81	92	60
p-Anisic		–	–	93	61
o-Anisic		1.0	56–71	94	62
p-Isopropyloxybenzoic		–	72	95	63
2,4,6-Trimethylbenzoic, (Mesitoic)		45	74.2	91	58

(*continued*)

TABLE II, 3. (*continued*)

Acid	RX	mmole	% yield	Ref.	Note
2,4,6-Trimethylbenzoic, (Mesitoic)		–	–	19	59
α-Phenyl-o-toluic, (o-Benzylbenzoic)		5	–	96	64
4-Benzyloxy-*m*-anisic		–	–	105	74
p-Cyclohexyloxybenzoic		–	77	95	65
1-Naphthoic		3.5	93	108	–
1-Naphthoic		18.4	82.4	103	78
2-Naphthoic		8.1	73	97	–
2-Naphthoic		2.7	70	98	66
2-Biphenylcarboxylic		5	85	99	67
2-Biphenylcarboxylic		20	94	100	79
2-Biphenylcarboxylic		31	60	101	68
2-Biphenylcarboxylic		50	60	102	69
7-Methyl-4-indancarboxylic		–	64	103	–
4-(Ethylthio)butyric	$C_2H_5S(CH_2)_3Br$	4.6	53	91	70

[6g]M. K. Phibbs and B. de B. Darwent, J. Chem. Phys., *18*, 679 (1950).
[6h]B. Riegel and Y. C. Liu, J. Org. Chem., *16*, 1610 (1951).
[6i]C. J. Collins, J. Am. Chem. Soc., *73*, 1038 (1951).
[6j]C. Baret and L. Pichat, Bull. soc. chim. France, *18*, 580 (1951).
[6k]D. L. Williams and A. R. Ronzio, J. Org. Chem., *18*, 489 (1953).
[6l]A. Darvnieks, Chem. Tech. (Berlin), *13*, 149 (1942).
[7]H. Gilman and A. Wood, J. Am. Chem. Soc., *48*, 806 (1926).
[8]H. Gilman, P. D. Wilkinson, W. P. Fishel and C. H. Meyers, J. Am. Chem. Soc., *45*, 150 (1923).
[9]H. Gilman, E. A. Zoellner and J. B. Dickey, J. Am. Chem. Soc., *51*, 1576 (1929).
[10]R. Macdonald and B. E. Baker, Can. J. Chem., *31*, 517 (1953).
[11]M. B. Neyman, A. F. Lukovnikov and B. Z. Iofa, Doklady Akad. Nauk S.S.S.R., *78*, 493 (1951).
[12]M. L. Sherrill, J. Am. Chem. Soc., *52*, 1982 (1930).
[13]V. Du Vigneaud, W. G. L. Verly, J. E. Wilson, J. R. Rachele, C. Ressler and J. M. Kinney, J. Am. Chem. Soc., *73*, 2782 (1951).
[14]S. Bergström, B. Borgström and M. Rottenberg, Acta Physiol. Scand., *25*, 120 (1952).
[15]D. Y. Barker and J. E. Christian, J. Am. Pharm. Assoc., Sci. Ed., *44*, 105 (1955).
[16]*Organic Syntheses*, Vol. 21, Wiley, New York, 1941, p. 77.
[17]A. A. Bothner-By and J. Bigeleisen, J. Chem. Phys., *19*, 755 (1951); W. H. Stephens, J. M. Pepper and M. Lounsbury, J. Chem. Phys., *20*, 192 (1952).
[18]P. Pfeiffer and W. Loewe, J. prakt. Chem., *147*, 293 (1937).
[18a]L. T. Hallett, Ind. Eng. Chem., Anal. Ed., *14*, 956 (1942).
[19]W. H. Stevens and R. W. Attree, Can. J. Research, *27B*, 807 (1949).
[20]A. McCoubrey and N. K. Iyengar, J. Chem. Soc., *1951*, 3430.
[21]A. Guyer, A. Bieler and E. Hardmeier, Helv. Chim. Acta, *20*, 1462 (1937).
[22]C. W. Bills and A. R. Ronzio, Atomic Energy Commission Report, AECU-1005; Nuc Sci. Abstracts, *5*, 585 (1951).
[22a]A. V. Logan and N. R. Odell, Weeds, *2*, 135 (1953).
[23]T. A. Geissman, Atomic Energy Commission Report, UCRL-1233; Nuc. Sci. Abstracts, *5*, 4722 (1951).
[24]D. Jerchel, H. Becker and K. Schmeiser, Z. Naturforsch., *8b*, 294 (1953).
[25]H. Schmid and K. Banholzer, Helv. Chim. Acta, *37*, 1706 (1954).
[26]F. Bergel, V. C. E. Burnop and J. A. Stock, J. Chem. Soc., *1955*, 1223.
[27]H. Pines and R. W. Myerholtz, Jr., J. Am. Chem. Soc., *77*, 5392 (1955).
[28]K. Bernhard, J. P. Vuilleurnier and G. Brubacher, Helv. Chim. Acta, *38*, 1438 (1955).
[29]M. M. Koton, T. M. Kiseleva and M. I. Bessonov, Doklady Akad. Nauk S.S.S.R., *96*, 85 (1954).
[30]C. Huggett, R. T. Arnold and T. I. Taylor, J. Am. Chem. Soc., *64*, 3043 (1942).
[30a]D. M. Hughes, R. Ostwald and B. M. Tolbert, J. Am. Chem. Soc., *74*, 2434 (1952).
[31]P. T. Adams, R. E. Selff and B. M. Tolbert, J. Am. Chem. Soc., *74*, 2416 (1952).
[32]W. Lamprecht and H. Rehberg, Hoppe Seyler's Z. physiol. Chem., *301*, 46 (1955).
[33]C. Baret and L. Pichat, Bull. soc. chim. France, *18*, 580 (1951).

[34]S. Weinhouse, G. Medes and N. F. Floyd, J. Biol. Chem., *166*, 691 (1946).
[35]J. D. Cox, H. S. Turner and R. J. Warne, J. Chem. Soc., *1950*, 3167.
[36]M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 178.
[37]E. M. Schantz and D. Rittenberg, J. Am. Chem. Soc., *68*, 2109 (1946).
[38]J. D. Roberts, R. E. McMahon and J. S. Hine, J. Am. Chem. Soc., *72*, 4237 (1950).
[39]T. I. Andrianova, E. A. Andreev and O. M. Sokolova, Doklady Akad. Nauk S.S.S.R., *88*, 677 (1953); Chem. Abstracts, *48*, 2565 (1954); AEC-tr-1827; Nuc. Sci. Abstracts, *7*, 3057 (1953).
[40]R. W. Schayer, J. Am. Chem. Soc., *74*, 2441 (1952).
[41]S. Ruben, M. B. Allen and P. Nahinsky, J. Am. Chem. Soc., *64*, 3050 (1942).
[42]N. S. Olson, A. Hemingway and A. O. Nier, J. Biol. Chem., *148*, 611 (1943).
[43]J. Anatol and J. Coursaget, Compt. rend., 233, 488 (1951).
[44]J. Bell and R. I. Reed, J. Chem. Soc., *1952*, 1383.
[45]A. V. Logan and J. Murray, J. Am. Chem. Soc., *74*, 2436 (1952).
[46]H. S. Anker, J. Biol. Chem., *176*, 1333 (1948).
[47]R. Muxart, P. Daudel, C. Rigolet and R. Melet, Bull. soc. chim. France, *19*, 577 (1952).
[48]B. Schepartz and S. Gurin, J. Biol. Chem., *180*, 663 (1949).
[49]J. M. Buchanan, A. B. Hastings and F. B. Nesbett, J. Biol. Chem., *150*, 413 (1943).
[50]R. Ostwald, P. T. Adams and B. M. Tolbert, J. Am. Chem. Soc., *74*, 2425 (1952).
[51]A. S. Gordon and S. Heimel, J. Am. Chem. Soc., *73*, 2942 (1951).
[52]M. Kleiber, A. L. Black, M. A. Brown and B. M. Tolbert, J. Biol. Chem., *203*, 339 (1953).
[53]R. J. Speer, A. Roberts, M. Maloney and H. R. Mahler, J. Am. Chem. Soc., *74*, 2444 (1952).
[54]P. Nahinsky, C. N. Rice, S. Ruben and M. D. Kamen, J. Am. Chem. Soc., *64*, 2299 (1942).
[55]B. A. Fries and M. Calvin, J. Am. Chem. Soc., *70*, 2235 (1948).
[56]A. F. Lukownikov, M. B. Neyman, A. A. Bag, L. M. Rodionova, I. S. Samoukina and N. V. Bliznyak, Doklady Akad. Nauk S.S.S.R., *88*, 297 (1953); Nuc. Sci. Abstracts, *7*, 3054 (1953): AEC-tr-1829.
[57]P. Nahinsky and S. Ruben, J. Am. Chem. Soc., *63*, 2275 (1941).
[58]G. Medes, S. Weinhouse and N. F. Floyd, J. Biol. Chem., *157*, 35 (1945).
[59]M. Kleiber, A. L. Black, M. A. Brown, J. Luick, C. F. Baxter and B. M. Tolbert, J. Biol. Chem., *210*, 239 (1954).
[60]I. Gray, P. Adams and H. Hauptmann, Experientia, *6*, 430 (1950).
[61]M. J. Coon and S. Gurin, J. Biol. Chem., *180*, 1159 (1949).
[62]W. Reeve, D. H. Chambers and C. S. Prickett, J. Am. Chem. Soc., *74*, 5369 (1952).
[63]H. Hauptmann, P. T. Adams and B. M. Tolbert, J. Am. Chem. Soc., *64*, 2423 (1952).
[64]I. Zabin and K. Bloch, J. Biol. Chem., *185*, 117 (1950).
[65]M. J. Coon, J. Biol. Chem., *187*, 71 (1950).
[66]M. J. Coon and N. S. B. Abrahamsen, J. Biol. Chem., *195*, 805 (1952).
[67]L. Pichat, C. Baret, M. Audinot, M. Herbert and J. Lambin, *Radioisotope Conference 1954*, Vol. I, Academic Press, New York, 1954, p. 245; C. Baret and L. Pichat, Bull. soc. chim. France, *18* (5), 580 (1951).

[68]R. J. Speer, D. R. Carmody, Nucleonics, 7 (4), 46 (1950).
[69]E. O. Weinman, I. L. Chaikoff, W. G. Dauben, M. Gee and C. Entenman, J. Biol. Chem., *184*, 735 (1950).
[70]G. V. Isagulyants, E. A. Andreev and N. A. Kosolapova, Doklady Adak. Nauk S.S.S.R., *91*, 1123 (1953): Chem. Abstracts, *48*, 1252 (1954).
[71]R. P. Geyer and M. Cunningham, J. Biol. Chem., *184*, 641 (1950).
[72]J. L. Kalinsky and A. Weinstein, J. Am. Chem. Soc., *76*, 3730 (1954).
[73]S. Weinhouse, G. Medes and N. F. Floyd, J. Biol. Chem., *155*, 143, (1944).
[74]R. P. Geyer, L. W. Mathews and F. J. Stare, J. Biol. Chem., *180*, 1037 (1949).
[75]B. Bloom, I. L. Chaikoff and W. O. Reinhardt, Am. J. Physiol., *166*, 451 (1951).
[76]J. C. Harris, R. E. Kamp and W. H. Yanko, A. S. T. M. Bull., *170*, 82 (1950).
[77]E. Hines and A. Gemant, Science, *110*, 19 (1949).
[78]B. Borgström, Acta Physiol. Scand., *25*, 315 (1952).
[79]J. C. Harris, A. S. T. M. Bull., *158*, 49 (1949).
[80]R. O. Brady, J. Rabinowitz, J. Van Baalen and S. Gurin, J. Biol. Chem., *193*, 137 (1951).
[81]G. G. Smith and D. G. Ott, J. Am. Chem. Soc., *77*, 2342 (1955).
[82]D. G. Ott and G. G. Smith, J. Am. Chem. Soc., *77*, 2325 (1955).
[83]L. Li and W. H. Elliott, J. Am. Chem. Soc., *74*, 4089 (1952).
[84]C. J. Collins, J. Am. Chem. Soc., *73*, 1038 (1951).
[85]J. R. Catch, H. P. W. Huggill and A. R. Somerville, J. Chem. Soc., *1953*, 3028.
[86]R. J. Speer, D. R. Carmody, N. L. Humphries and A. Roberts, Nucleonics, 7 (4), 48 (1950).
[87]D. R. Buhler, C. H. Wang and B. E. Christensen, J. Am. Chem. Soc., *75*, 4336 (1953).
[88]W. H. Stevens and D. A. Holland, Science, *112*, 718 (1950).
[89]W. L. Carrick and A. Fry, J. Am. Chem. Soc., *77*, 4381 (1955).
[90]H. I. Hadler, J. Am. Chem. Soc., *77*, 1052 (1955).
[91]A. E. Almond, A. M. Burditt, D. E. Douglas and J. Eccles, Can. J. Chem., *33*, 679 (1955).
[92]J. C. Reid and H. B. Jones, J. Biol. Chem., *174*, 427 (1948).
[93]S. A. Brown and A. C. Neish, Can. J. Biochem. and Physiol., *33*, 948 (1955).
[94]F. Weygand, A. Becher, D. Feldmann and A. O. Grosskinsky, Hoppe Seyler's Z. physiol. Chem., *292*, 138 (1953).
[95]J. B. Brierley and A. McCoubrey, Brit. J. Pharmacol., *8*, 366 (1953).
[96]W. H. Stevens and D. A. Crowder, Can. J. Chem., *32*, 792 (1954).
[97]C. Heidelberger, P. Brewer and W. G. Dauben, J. Am. Chem. Soc., *69*, 1389 (1947).
[98]R. V. Phillips, L. W. Trevoy, L. B. Jaques and J. W. T. Spinks, Can. J. Chem., *30*, 844 (1952).
[99]C. Heidelberger and H. S. Rieke, Cancer Research, *11*, 640 (1951).
[100]A. S. Harris, E. N. White and D. McNeil, J. Chem. Soc., *1955*, 4216.
[101]F. E. Ray and R. C. Geiser, Cancer Research, *10*, 616 (1950).
[102]H. R. Gutman and J. H. Peters, Cancer Research, *13*, 415 (1953).
[103]W. G. Dauben, J. Org. Chem., *13*, 313 (1948).
[104]W. R. Vaughan and D. I. McCane, J. Am. Chem. Soc., *76*, 2504 (1954).
[105]K. Freudenberg and M. Reichert, Ber., *87*, 1834 (1954).

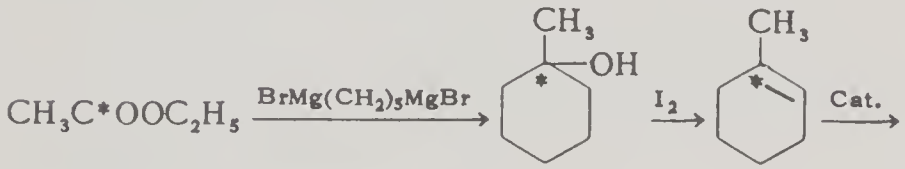
[106]B. Riegel and H. Wittcoff, J. Am. Chem. Soc., *68*, 1915 (1946); K. Freudenberg, H. Fikenstcher and M. Harder, Ann., *441*, 178 (1925).

[107]O. Simamura, N. Inamoto and T. Suehiro, Bull. Chem. Soc. Japan, *27*, 221, (1954); through Chem. Abstracts, *49*, 7494 (1955); B. H. Shoemaker and C. E. Boord, J. Am. Chem. Soc., *53*, 1505 (1931).

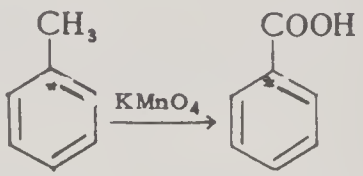
[108]P. T. Adams and D. Kritchevsky, Atomic Energy Commission Report, UCRL-1262: Nuc. Sci. Abstracts, *5*, 4723 (1951).

[109]G. W. Pearce and J. A. Jensen, J. Agr. Food Chem., *1*, 776 (1953).

BENZOIC-1-C^{14} ACID



(a) (b)



(c) (d)

M. Fields, M. A. Leaffer, S. Rothchild and J. Rohan, J. Am. Chem. Soc., *74*, 5498 (1952); M. Fields, M. A. Leaffer and J. Rohan, Science, *109*, 35 (1949).

A. Procedure

(a) *1-Methylcyclohexanol-1-C^{14}* (Note 1). A Grignard reagent is prepared from 8.75 g. (360 mmoles) of magnesium and 39.4 g. (171 mmoles) of freshly distilled pentamethylene bromide[1] in 215 ml. of absolute ether (Note 2). Ethyl acetate-1-C^{14} (5.04 g., 57.2 mmoles) in 60 ml. of dry ether is added slowly to the vigorously stirred suspension of Grignard reagent cooled in an ice-bath. The mixture is stirred overnight at room temperature, then refluxed for 1 hour. The complex is decomposed with saturated ammonium chloride solution, and the aqueous phase is extracted with several portions of ether. The combined extract, washed with water until neutral, is dried over calcium sulfate and fractionally distilled; yield 5.27 g. (46.2 mmoles), b.p. 62–66° (25 mm.) (Note 3).

(b) *1-Methylcyclohexene-1-C^{14}* (Note 4). To 5.27 g. of 1-methylcyclohexanol-1-C^{14}, contained in a small flask attached to a 9-in. Podbielniak column, is added a small crystal of iodine. Dehydration proceeds smoothly, and a mixture of water and methylcyclohexene slowly distills at a bath

temperature of 135–140°. The product is separated from the water layer, dried over calcium hydride and distilled; yield 2.11 g. (39.5% based on ethyl acetate), b.p. 103–106°, n_D^{20} 1.4495 (Note 5).

(c) *Toluene-1-C*^{14}. The dehydrogenation apparatus (Note 6) consists of a pot, a heated catalyst tube (90 cm. long) attached to a side arm equipped with a stopcock (to allow the product to be recycled), and a reflux condenser. A 3-l. gas burette, connected to the condenser, is used to measure the volume of evolved hydrogen over water. The catalyst tube is charged with a 70-cm. column of pelleted-dehydrogenation catalyst (Note 7) and is heated slowly to 450°, while a gentle stream of dry hydrogen is passed through the system. After 20 hours, the hydrogen flow is interrupted while the system is evacuated (0.1 mm.) until no more water is evolved; then hydrogen is readmitted to restore atmospheric pressure. 1-Methylcyclohexene-1-C^{14} (2.11 g., 22 mmoles) is distilled slowly through the catalyst at 450°. The first rapid hydrogen evolution practically ceases after a second or third cycle over the catalyst (Note 8). The yield of pure product is 1.91 g. (95%), n_D^{29} 1.4908 (Note 9).

(d) *Benzoic-1-C*^{14} *Acid* (Note 10). Toluene-1-C^{14} is oxidized with 7.80 g. of potassium permanganate in 96 ml. of water at 95°, and the manganese dioxide is reduced with sulfur dioxide. The yield of acid is 1.87 g. (15.4 mmoles), 74%, m.p. 122.6–123.0°, and the molar specific activity is unchanged from that of the starting carbon-C^{14} dioxide.

B. Notes

1. The procedure is a modification of the method of Grignard.[2] A considerable excess of Grignard reagent materially increases the yield of carbinol.

2. The Grignard reagent separates from ether as an oil. Dissolution of the reagent in an ether-benzene mixture does not increase the yield of carbinol and makes the isolation more difficult.

3. This degree of purity is suitable for dehydration. The last traces of high activity material remaining in the distillation apparatus and high boiling residue in small-scale preparations are removed by continuing the distillation with a stripping technique using carrier.

4. The procedure is an adaptation of the method of Mosher[3] for dehydrating tertiary alcohols containing the cyclohexane ring.

5. The earlier paper reported yields of 60–70% for the condensation and 80–85% for the dehydration. The refractive index, n_D^{20} 1.4495, agrees well with the literature[4] value, n_D^{20} 1.4497.

6. Use of a similar apparatus (Figure II, 7) is described in detail by Ruzicka.[5]

7. The Universal Oil Products "Dehydrogenation Catalyst" (a base metal dehydrogenation catalyst) produces a quantitative evolution of hy-

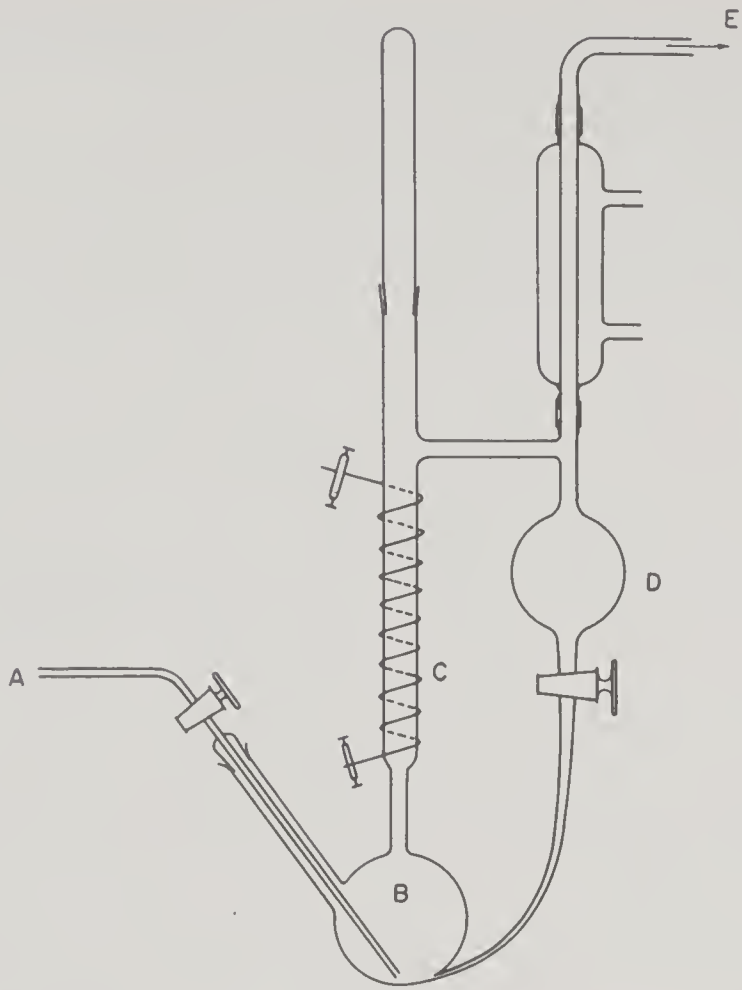


Fig. II, 7. Apparatus for cyclic catalytic operations in the vapor phase (L. Ruzicka and M. Stoll). A, gas inlet; B, distillation pot; C, electrically heated catalyst column; D, receiver; E, outlet to vacuum manifold or gas burette.

drogen with formation of a pure toluene. Dehydrogenation over 30% platinum or palladium-on-asbestos results in only 65–80% evolution of the theoretical quantity of hydrogen and formation of a mixture of products.

8. In all, 926 ml. S.T.P. (41.5 mmoles) of dry hydrogen is collected, calculated 985 ml. (44.0 mmoles).

9. The yield figure includes a small quantity of toluene isolated from the hot catalyst bed by evacuation at 0.1 mm. The refractive index of an authentic specimen is n_D^{20} 1.4892. The earlier paper reported yields of 90–95%.

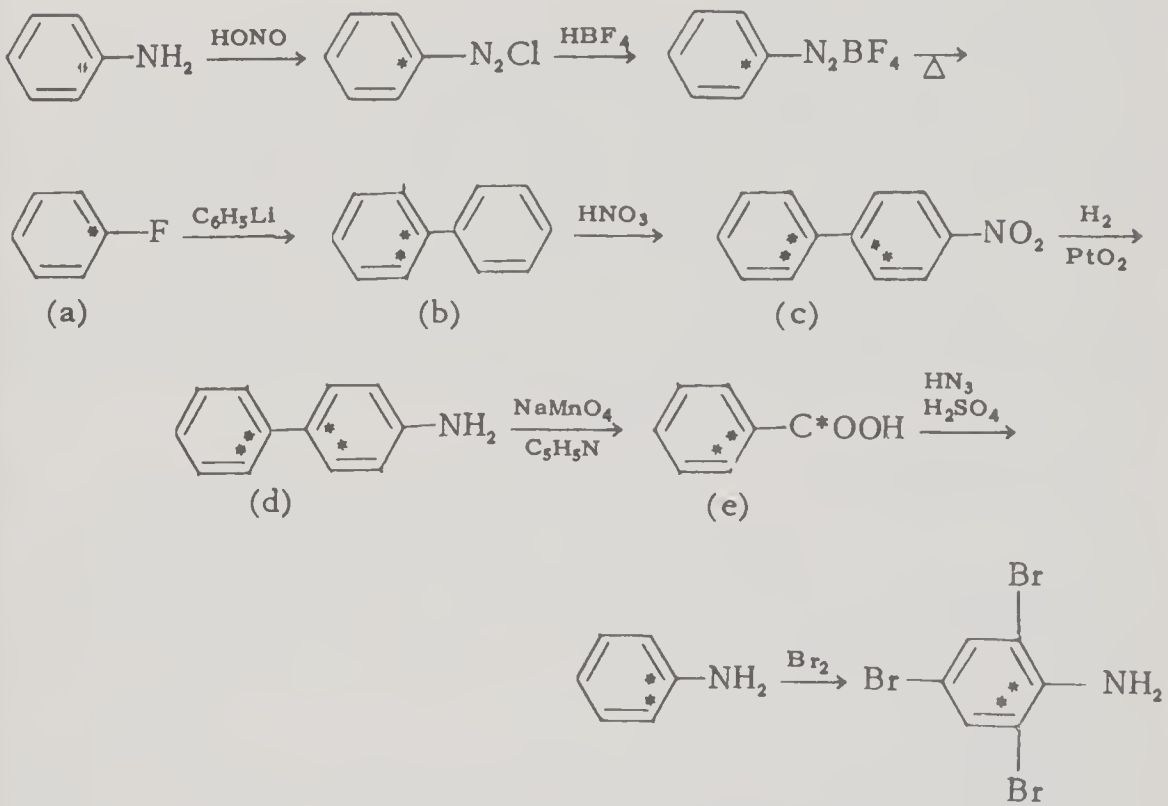
10. The procedure is adapted from the method of Ullmann;[6] also see benzoic-1,2,3,4-$C^{14}_{1/4}$ acid.

C. Other Preparations

In a similar manner from ethyl formate-C^{14} and 1,5-dibromo-3-methylpentane[7,1] is prepared[8] 4-methylcyclohexanol-1-C^{14}; yield 48%, b.p. 80° (16 mm.).

[1]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, pp. 428 and 99.
[2]V. Grignard and G. Vignon, Compt. rend., *144*, 1358 (1907).
[3]W. A. Mosher, J. Am. Chem. Soc., *62*, 552 (1940).
[4]K. Auwers and P. Ellinger, Ann., *387*, 220 (1912).
[5]L. Ruzicka and M. Stoll, Helv. Chim. Acta, *7*, 90 (1924).
[6]F. Ullmann and J. B. Uzbachian, Ber., *36*, 1797 (1903).
[7]N. J. Leonard and Z. W. Wicks, J. Am. Chem. Soc., *68*, 2402 (1946).
[8]R. Dische and D. Rittenberg, J. Biol. Chem., *211*, 199 (1954).

BENZOIC-$C^{14}_{1/1}$-1,2-$C^{14}_{1/2}$ ACID



E. F. Jenny and J. D. Roberts, Helv. Chim. Acta, *38*, 1248 (1955).

A. Procedure (Note 1)

(a) *1-Fluorobenzene-1-C^{14}* (Note 2). To a stirred solution of 8.5 ml. of water, 10.3 ml. of concentrated hydrochloric acid and a one-half portion of aniline hydrochloride (12.5 g. total), maintained at $-5°$, is added dropwise a solution of 7.5 g. of sodium nitrite in 9.4 ml. of water. When about one-half of the total aniline hydrochloride has been diazotized, 0.040 g. of aniline-1-C^{14} hydrochloride is added, and the reaction is com-

pleted with the remaining portion of aniline hydrochloride (Note 3). The stirred diazonium salt solution is diluted at −7° with 19.9 g. of aqueous 44.5% fluoboric acid at 0° (Note 4). The magma of benzenediazonium-1-C^{14} fluoborate is stirred for 20–30 minutes and filtered, the solid being washed with ice-water, methanol and ether (Note 5).

The air-dried salt is placed in a large flask, connected by wide-bore tubing to three traps cooled with ice-salt, and is heated gently at one point near its surface with a small flame to initiate the decomposition. Heat is applied vigorously at the end until no more fumes of boron fluoride are evolved (Note 6). The combined distillate is washed with 10.0% sodium hydroxide solution and water, dried over calcium chloride and distilled, the still being scavanged with 3.0 g. of carrier; yield 10.1 g., b.p. 81–82°.

(b) *Biphenyl-1,2-*$C^{14}_{1/2}$ (Note 1). To a stirred suspension of 1.9 g. (274 mmoles) of lithium chips (1–2 $mm.^3$) and 3.5 ml. of absolute ether under nitrogen is added dropwise 25.7 g. (164 mmoles) of bromobenzene in 80 ml. of ether at a rate to maintain gentle refluxing. When the lithium disappears after 40 minutes of heating, the phenyllithium solution is treated with the 1-fluorobenzene-1-C^{14} (105 mmoles) in 15 ml. of ether. The mixture is heated for 15 minutes, stored for 24 hours, chilled and slowly diluted with 50 ml. of ice water. The ether extract is washed with water, dried over sodium sulfate and fractionally distilled through a column; yield 8.40 g., b.p. 135° (20 mm.) (Note 7).

(c) *4-Nitrobiphenyl-1,1′,2,2′-*$C^{14}_{1/4}$ (Note 8). To a solution of 8.1 g. of biphenyl-1,2-$C^{14}_{1/2}$ in 8.1 ml. of glacial acetic acid at 80° is added dropwise 5.0 ml. of concentrated nitric acid. The mixture is heated at 90° for 30 minutes and cooled to 0° to crystallize the product, which is collected, washed with water and dried in a desiccator; yield 6.4 g., m.p. 108–115° (Notes 9 and 10).

2-Nitrobiphenyl-1,1′,2,2′-$C^{14}_{1/4}$. The above filtrate is extracted with ether, and the extract is washed with 20% potassium carbonate solution and water, dried over sodium sulfate and fractionally distilled through a column; yield 3.0 g., b.p. 90–100° (2 mm.), m.p. 36–37° (Notes 9 and 11).

(d) *4-Biphenylamine-1,1′,2,2′-*$C^{14}_{1/4}$, *(*C^{14}_1*-Xenylamine).* 4-Nitrobiphenyl-1,1′,2,2′-$C^{14}_{1/4}$ is hydrogenated at 10 atmospheres in 70 ml. of 95% alcohol over 0.140 g. of platinum oxide, the up-take stopping in 10 minutes. The filtered solution is evaporated at 40° (20 mm.), giving 5.4 g. of crystalline product (Notes 8 and 12).

(e) *Benzoic-*$C^{14}_{1/1}$*-1,2-*$C^{14}_{1/2}$ *Acid.* To a stirred boiling solution of 5.4 g. of 4-biphenylamine-1,1′,2,2′-$C^{14}_{1/4}$ and 2.6 g. of 2-biphenylamine-1,1′,2,2′-$C^{14}_{1/4}$ in 300 ml. of pyridine is added, dropwise during 30 minutes, a solution of 100 g. of sodium permanganate trihydrate in 200 ml. of water. The heating is continued overnight, and the mixture is filtered hot, the man-

ganese dioxide being washed thoroughly with hot water. The filtrate is evaporated at 60° (20 mm.), and the residue is acidified with dilute sulfuric acid. The product is extracted into ether solution, which is washed with water, dried over sodium sulfate and evaporated. The crude product (1.6 g.), obtained by sublimation at 100° (20 mm.), is purified by three recrystallizations from alcohol-water and resublimation, m.p. 121–122°. The molar specific activity is 73.6 ± 0.9% of that of the starting material (Note 13).

B. Notes

1. These preparations are incidental to a tracer study of the mechanism of biphenyl formation by the method of Wittig.[1]

2. The procedure is an adaptation of that of Flood.[2]

3. The diazotization is stopped when a positive test for nitrous acid is obtained with potassium iodide-starch paper.

4. Directions are given by Flood[2] for preparing fluoboric acid.

5. The presence of moisture affects the stability of the salt.

6. The exit fumes may be absorbed in a trap containing soda solution. The last traces of product may be removed from the reaction flask by applying gentle suction to the receiving train.

7. An aliquot, recrystallized twice from 90% alcohol and sublimed at 140° (20 mm.), melted at 70–70.5°.

8. The procedure is an adaptation of that of Morgan.[3]

9. Degradation studies indicate that the isotopic distribution is essentially statistical.

10. Pure 4-nitrobiphenyl melts at 114–114.5° from alcohol, b.p. 223–224° (30 mm.).

11. Pure 2-nitrobiphenyl melts at 37° from alcohol, b.p. 160–166° (4 mm.).

12. Pure 4-biphenylamine melts at 50–52° from alcohol-water, b.p. 166° (5 mm.).

A similar reduction of the 2-nitrobiphenyl-1,1′,2,2′-$C^{14}_{1/4}$ at room temperature gives 2.6 g. of crystalline 2-biphenylamine-1,1′,2,2′-$C^{14}_{1/4}$. Pure 2-biphenylamine melts at 49–50° from alcohol-water, b.p. 145–148° (5 mm.).

13. The calculated value based on statistical distribution is 75.0%, in the absence of isotope effects. Directions are given by Jenny and Roberts for degrading the acid to aniline-1,2-$C^{14}_{1/2}$, by the Schmidt reaction. Bromination,[4] for assay purposes, gave crystalline 2,4,6-tribromoaniline-1,2-$C^{14}_{1/2}$, m.p. 118–118.5°, having a molar specific activity 49.8 ±

0.6% of that of the starting material (calc. 50.0%, in the absence of isotope effect).

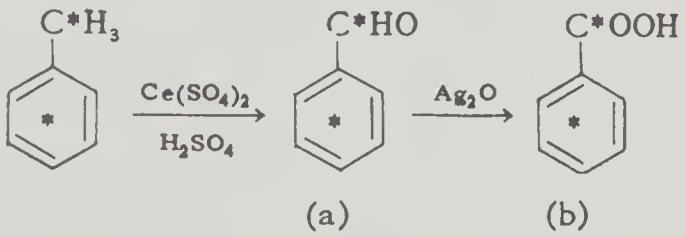
[1]G. Wittig, G. Pieper and G. Fuhrmann, Ber., 73, 1193 (1940); G. Wittig, Angew. Chem., 66, 10 (1954).

[2]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 295.

[3]G. T. Morgan and L. P. Walls, J. Soc. Chem. Ind. (London), 49, 15T (1930).

[4]N. D. Cheronis and J. B. Entrikin, *Semimicro Qualitative Organic Analysis*, Crowell, New York, 1947, p. 179.

BENZOIC-C^{14}-1,2,3,4-$C^{14}_{1/4}$ ACID



A. E. H. Kilner, H. S. Turner and R. J. Warne, *Radioisotope Conference, 1954*, Vol. II, Academic Press, New York, 1954, p. 23.

A. Procedure (Note 1)

(a) *Benzaldehyde-C^{14}-1,2,3,4-$C^{14}_{1/4}$* (Note 1). A sealed flask containing 3 mmoles of toluene-(α,1), (α,2), (α,3), (α,4)-$C^{14}_{2/4}$ and 5–10% excess of ceric sulfate in 9*N* sulfuric acid is rotated at an angle of 30°, while partly immersed in a bath at 90° for 3–4 hours. The mixture is extracted with ether, and the ether solution is washed with dilute alkali (Note 2), dried and evaporated. The residue is added to an excess of buffered semicarbazide hydrochloride. The benzaldehyde-C^{14}-1,2,3,4-$C^{14}_{1/4}$ semicarbazone (yield 76%) (Note 3) is hydrolyzed with 9 *N* sulfuric acid, the product being continuously removed by steam distillation. The distillate is extracted with ether, and the extraction solvent is removed through a column.

(b) *Benzoic-C^{14}-1,2,3,4-$C^{14}_{1/4}$ Acid.* The aldehyde is oxidized with freshly precipitated silver oxide.[1]

B. Notes

1. The procedure is an adaptation of a method described in an early German patent, DRP. 158,609 (1905). The method is believed to be more

satisfactory for use with high activity material at the 10-mmole level than is the usual alkaline permanganate oxidation.

2. The alkaline solution contains a 22% yield of steam-volatile acids, including a 1–2% yield ot benzoic-C^{14}-1,2,3,4-$C^{14}_{1/4}$ acid (isotope dilution analysis) and a trace of C^{14}-anthraquinone.

3. Isotope dilution analysis indicates a yield of 80%.

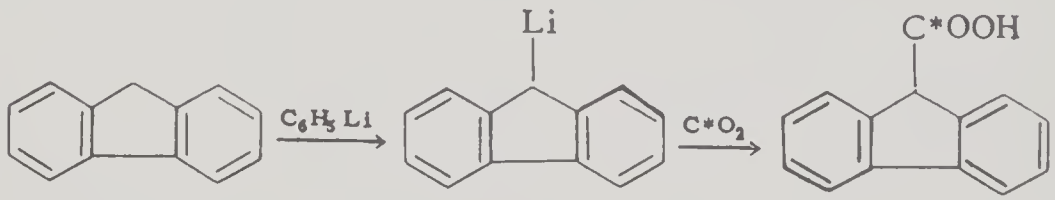
C. Other Preparations

Benzaldehyde-$C^{14}_{1/1}$-1,2,3,4-$C^{14}_{1/4}$ and benzoic-$C^{14}_{1/1}$-1,2,3,4-$C^{14}_{1/4}$ are prepared in the same manner from toluene-α,1,2,3,4-$C^{14}_{1/5}$.

[1]*Organic Syntheses*, Vol. 33, Wiley, New York, 1953, p. 94.

9-FLUORENECARBOXYLIC-C^{14} ACID

METHOD I

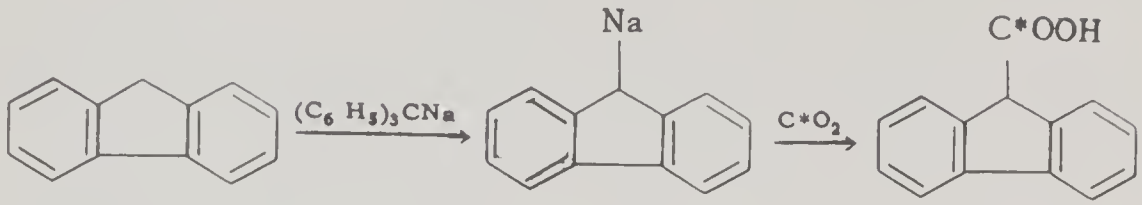


A. S. Harris, E. N. White and D. McNeil, J. Chem. Soc., *1955*, 4216.

A. Procedure

To 8.4 g. of fluorene under nitrogen is added a solution of phenyllithium, prepared[1] from 8 g. of bromobenzene and 0.8 g. of lithium in 50 ml. of absolute ether. The mixture is frozen with liquid nitrogen, and the flask is evacuated to a few microns. The stirred, orange-colored mixture is carbonated at −60° with 1.24 mmoles of carbon-C^{14} dioxide, followed by 18.8 mmoles of carbon dioxide. The mixture is stirred for several hours to ensure complete reaction, then is acidified with 2 *N* sulfuric acid. The ether solution is separated and extracted with sodium carbonate solution which is acidified to give the product; yield 4.01 g. (95%), m.p. 228–229°.

METHOD II



C. J. Collins, J. Am. Chem. Soc., 70, 2418 (1948).

A. Procedure (Note 1)

The carbonation apparatus consists of a carbon-C^{14} dioxide generator (with a nitrogen inlet) connected by a stopcock to a spiral trap (cooled with Dry Ice), a manometer, a spiral trap (cooled with liquid nitrogen), and a reaction flask, which is fitted with a magnetic stirrer, a connection to a vacuum manifold and an exhaust to the atmosphere through a Drierite tube, a barium hydroxide bubble trap and a soda lime tube (Note 2).

Barium carbonate-C^{14} (0.100 g.) is decomposed in an atmosphere of dry nitrogen by the addition of 5–8 ml. of 5 *N* perchloric acid. Nitrogen is passed through the system for 15 minutes, and the carbon-C^{14} dioxide, dried by passage through the Dry Ice trap at $-50°$ to $-55°$ (Note 3), is frozen in the liquid nitrogen trap. After 15 minutes, the acid mixture is heated gently to expel the last of the carbon-C^{14} dioxide. To 0.150 g. of fluorene, under nitrogen in the reaction flask, is added 3.5 ml. of a stock triphenylmethylsodium solution,[2] prepared from 2.0 g. of sodium, 5.0 g. of chlorotriphenylmethane, 40 ml. of absolute ether and 40 ml. of anhydrous benzene, according to the procedure of Bachmann and Wiselogle[2] (Note 4). The mixture is stirred for 3 minutes (Note 5), then frozen with liquid nitrogen, and the flask and two traps are evacuated to less than 1 mm. The carbon dioxide is distilled into the reaction flask, the flask is isolated from the system, and the mixture is thawed until the magnetic stirrer becomes operative. Completion of the reaction is indicated by a zero pressure, when the mixture is cooled to Dry Ice temperature. Nitrogen is admitted to the system, and the mixture is hydrolyzed with 10 ml. of water. The ether-benzene layer is separated and extracted with two 5-ml. portions of water, the aqueous solutions then being washed with two 3-ml. portions of ether (Note 6). The combined aqueous solutions are acidified with 10 ml. of *N* hydrochloric acid and extracted with four 4-ml. portions of ether. The ether solution is washed with 4 ml. of water, then extracted with three 4-ml. portions of saturated sodium bicarbonate solution. The alkaline solution is washed with 3 ml. of ether and acidified with 10 ml. of 6 *N* hydrochloric acid. Extraction with ether and evaporation yields 0.073–0.080 g. of product, m.p. 222–226° (Note 7).

B. Notes

1. The procedure is a modification of the method of Burtner and Cusic.[3]

2. A diagram of the system is presented by Collins. The generator consists of a small flask equipped with an addition funnel and nitrogen inlet, having a two-way stopcock arranged so that gas cannot be swept out through the addition tube.

3. The cooling bath is controlled by adjusting the amount of Dry Ice in a chloroform-carbon tetrachloride solution. A significant amount of carbon dioxide is retained at Dry Ice temperature.

4. A preparation of triphenylmethylsodium is described also by Renfrow and Hauser.[4]

5. The color changes from deep red to light orange.

6. A diagram of an extraction apparatus designed to prevent mechanical loss and minimize laboratory contamination is given by Collins. Use of a continuous liquid-liquid extractor also conveniently achieves this end; see *p*-aminobenzoic-C^{14} acid.

7. Consistent yields of 69–75% are obtained, the best being 86%.

C. Other Preparations

By the procedure of Method II, starting with the appropriate hydrocarbon, are prepared a number of homologues:

1-Methyl-9-fluorenecarboxylic-C^{14} acid,[5] m.p. 222.5–223° after three recrystallizations from benzene.

3-Methyl-9-fluorenecarboxylic-C^{14} acid,[6] m.p. 230–233°. The yield is 96% when carbon dioxide is in excess.

3-Methoxy-9-fluorenecarboxylic-C^{14} acid,[6] 90%, m.p. 190.3–191.2° after two crystallizations from toluene.

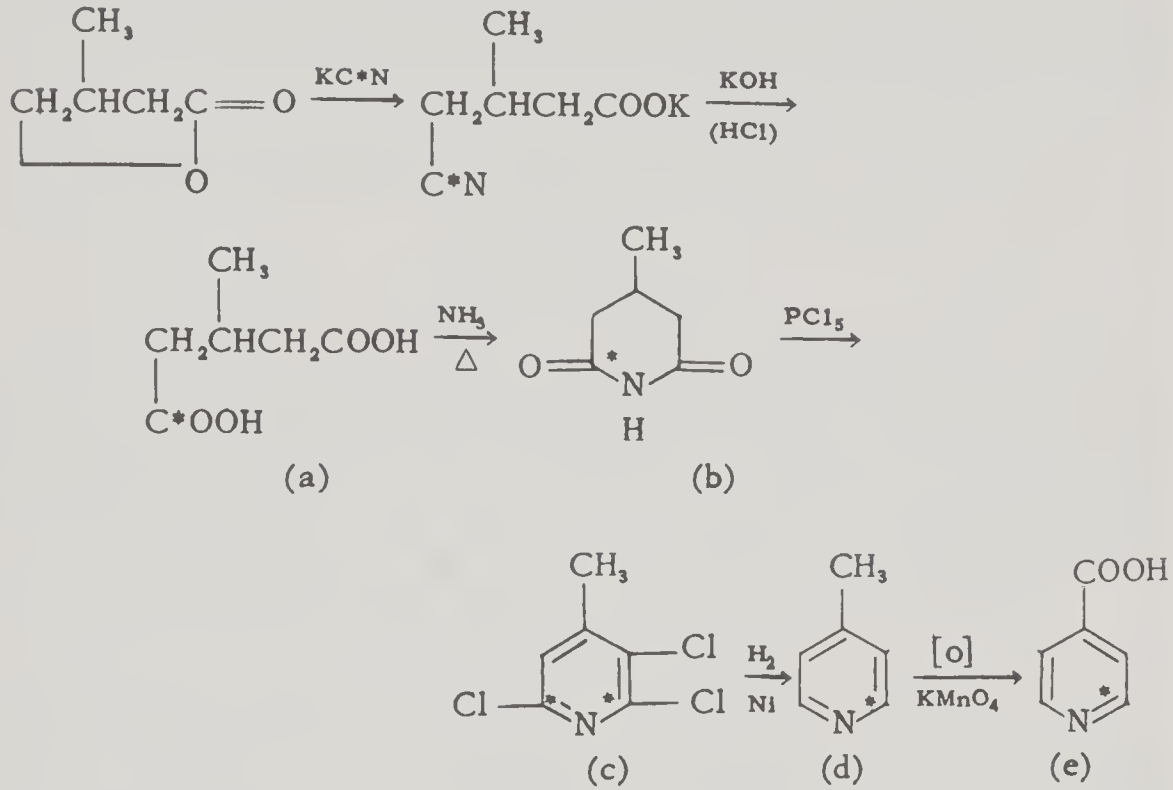
11*H*-Benzo[a]fluorene-11-carboxylic-C^{14} acid,[7] 83%, m.p. 218–221° (dec.) from benzene. Since the sodium salt of this acid is somewhat ether soluble, the yield is raised to nearly 100% by shortening the period of ether extraction of the alkaline solution, and by recovering the unreacted carbon dioxide.

11*H*-Benzo[b]fluorene-11-carboxylic-C^{14} acid,[8] 85%, m.p. 247–248° from benzene.

[1]G. Wittig, *Newer Methods of Preparative Organic Chemistry*, Interscience, New York, 1948, p. 576.
[2]W. E. Bachmann and F. Y. Wiselogle, J. Am. Chem. Soc., 58, 1943 (1936).
[3]R. R. Burtner and J. W. Cusic, J. Am. Chem. Soc., 65, 262 (1943).
[4]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 607.
[5]B. M. Benjamin and C. J. Collins, J. Am. Chem. Soc., 75, 402 (1953).
[6]C. J. Collins and B. M. Benjamin, J. Am. Chem. Soc., 75, 1644 (1953).
[7]C. J. Collins, D. N. Hess, R. H. Mayor, G. M. Toffel and A. R. Jones, J. Am. Chem. Soc., 75, 397 (1953).

[8]C. J. Collins, J. G. Burr, Jr., and D. N. Hess, J. Am. Chem. Soc., 73, 5176 (1951).

ISONICOTINIC-2-C^{14} ACID



L. Pichat, C. Baret and M. Audinot, Bull. soc. chim. France, (5) *21*, 88 (1954); L. Pichat, C. Baret, M. Audinot, M. Herbert and J. Lambin, *Radioisotope Conference, 1954*, Vol. I, Academic Press, New York, 1954, p. 245

A. Procedure (Note 1)

(a) *3-Methylglutaric-1-C^{14} Acid* (Note 2). A mixture of 0.400 g. of 3-methylbutyrolactone (Note 3) and 0.195 g. (3 mmoles) of dry potassium cyanide under dry nitrogen is shaken for 6 hours at 280° in a sealed tube (Note 4). The tube is cooled with Dry Ice, and the pale yellow contents are dissolved in 25 ml. of water and washed with ether. The aqueous solution is treated with 1.5 g. of potassium hydroxide and refluxed for 24 hours to hydrolyze the potassium 4-cyano-C^{14}-3-methylbutyrate. The mixture is adjusted to pH 3 with hydrochloric acid and continuously extracted with ether for 24 hours; yield 0.411 g. (94%).

(b) *4-Methylglutarimide-2-C^{14}*. The acid is placed in a tube equipped with an air condenser and a gas inlet reaching to the bottom (Note 5), and is heated at 150° (bath temperature) in a gentle stream of dry ammonia (Note 6) for 1.5 hours; then the temperature is raised to 270–280° for 30 minutes (Note 7). The apparatus walls are rinsed down with a few ml. of acetone, which is then evaporated on a water-bath. The inlet tube is replaced by a Dry Ice-cooled cold finger, and the product is collected by

sublimation at 100° (1 μ); yield 0.319 g. (84% based on potassium cyanide), m.p. 140–141°.

(c) *2,3,6-Trichloro-4-picoline-2,6-$C^{14}_{1/2}$* (Note 8). A mixture of the imide and phosphorus pentachloride (mmole ratio 1 : 3) is heated for 1 hour at 50° under a Dry Ice condenser (Note 9) and at 100° for 30 minutes. The mixture is cooled, diluted with water and continuously extracted with ether for 5 hours; yield 85% based on the imide (Note 10).

(d) *4-Picoline-2-C^{14}*. A mixture of 1 g. of Raney nickel (Note 11), 15 ml. of absolute ethanol and 0.800 g. of potassium hydroxide is shaken with hydrogen for 1 hour at room temperature. A solution of the crude trichloropicoline in 20 ml. of absolute alcohol is added, and the reaction is continued for 15 hours (Note 12). The alkaline mixture is vacuum-distilled into a trap containing 3 ml. of *N* sulfuric acid; then the alcohol is distilled back, leaving crude 4-picoline-2-C^{14} sulfate (Note 5). The free base is liberated by the addition of 6 *N* sodium hydroxide and vacuum-distilled.

(e) *Isonicotinic-2-C^{14} Acid.* A mixture of the picoline, 0.700 g. of potassium permanganate and 20 ml. of water is shaken and heated at 110° for 1 hour in a sealed tube. The cooled tube is opened, and the manganese dioxide is separated by filtration and thorough washing with boiling water. The combined solution is adjusted to pH 3.6 with hydrochloric acid and continuously extracted with ether for 48 hours; yield 0.129 g. (35% based on potassium cyanide).

B. Notes

1. The results here reported are representative of those obtained with nonisotopic potassium cyanide. A synthesis with 0.297 g. of potassium cyanide-C^{14}, which contained about 0.189 g. of potassium hydroxide, yielded in turn: 0.444 g. of impure 4-methylglutarimide-2-C^{14} and 0.058 g. of isonicotinic-2-C^{14} acid.

2. The procedure is an adaptation of that of Wislecenus.[1]

3. The starting material is prepared[2] from 3-methylglutaric acid[3] by heating the silver salt with iodine in the presence of quartz sand.

4. The yield is diminished if the reaction is conducted in the presence of air, or under vacuum, or at a lower temperature or for as long as 15 hours.

5. A diagram of the apparatus is presented in the journal reference.

6. Attempts to prepare the amide by heating the acid with urea were unsatisfactory.

7. At lower temperatures a mixture of acid amide and diamide is formed.

8. The method is essentially that of Crouch.[4]

9. Hydrogen chloride is evolved and the mass liquifies.

10. A sample recrystallized from aqueous-alcohol melts at 82–83°.
11. Palladium catalyst leads to the formation of 4-pipecoline.
12. Hydrogen absorption is complete in 3–4 hours.

[1]W. Wislecenus, Ber., *18*, 172 (1885); Ann., *233*, 101 (1886); E. E. Blaise, Compt. rend., *126*, 1153 (1898).

[2]S. S. Guha-Sircar, J. Chem. Soc., *1928*, 899.

[3]*Organic Syntheses*, Coll. Vol. III, Wiley, New York, 1955, p. 591.

[4]W. W. Crouch and H. L. Lochte, J. Am. Chem. Soc., *65*, 270 (1943); H. J. Den Hertog and E. Farenhorst, Rec. trav. chim., *67*, 380 (1948).

OXALIC-C_1^{14} ACID

$$CH_3CH_2C^*OOH \xrightarrow[NaOH]{KMnO_4} \begin{matrix} C^*OOH \\ | \\ COOH \end{matrix} \xrightarrow{Ca(OAc)_2} \left[\begin{matrix} C^*OO \\ | \\ COO \end{matrix}\right]^{=} Ca^{++}$$

R. J. Speer, A. Roberts, M. Maloney H. R. Mahler, J. Am. Chem. Soc., *74*, 2444 (1952).

A. Procedure (Note 1)

Calcium Oxalate-C_1^{14}. A vigorously stirred mixture of 20 mmoles of propionic-1-C^{14} acid, 49.2 g. of sodium hydroxide (Note 2), 15.7 g. of potassium permanganate and water (to 180 ml.) is heated quickly under reflux and maintained at 100° for 40 minutes. The mixture is cooled in an ice-bath, the excess oxidant is destroyed with 3% hydrogen peroxide, and the precipitate is removed and washed thoroughly with boiling water. The solution and washings are combined, acidified with acetic acid and swept with nitrogen to remove carbon-C^{14} dioxide. The mixture is treated with excess calcium acetate and digested, and the product is collected, washed and dried; yield 90%. The radiochemical yield is 80% (Note 3).

B. Notes

1. The procedure is adapted from that of Nahinsky;[1,2] also see Mahler.[3]

2. The oxidation of the starting material is followed by a slow oxidation of the product, diminishing with increasing alkalinity. Less than 0.4% of the oxalate is oxidized by permanganate in 2 *N* sodium hydroxide in 1 hour at 100°. The rate of propionate oxidation is increased by hydroxyl ion, varying approximately linearly above 2 *N* concentration.

The presence of a pronounced isotope effect in the oxidation of oxalic-C_1^{14} acid by three oxidants has been reported by Bunton.[4]

3. In 2 *N* sodium hydroxide, the C_2-C_3 bond in propionic acid is broken about 2.5 times as often as the C_1-C_2 bond. In 11 *N* sodium hydroxide,

the preferential C_2-C_3 rupture ratio rises[2,3] to 6.6–8.9. There is no exchange between oxalate and carbonate. The carbonate-oxalate ratio is unity.

C. Other Preparations

Calcium oxalate-C_1^{11} has been prepared by Nahinsky[2], and calcium oxalate-C_1^{14} has been prepared by Mahler[3] by oxidizing propionic-1-C^{14} acid, hydracrylic-1-C^{14} acid, lactic-1-C^{14} acid and pyruvic-2-C^{14} acid with permanganate. Oxidative mechanisms have been proposed.

Sodium oxalate-C_1^{14} has been formed[5] as a by-product (84% yield) in the condensation of pyruvic-2-C^{14} acid to methyldihydrotrimesic-1,3,5-C_3^{14} acid; see uvitic-1,3,5-C_3^{14} acid.

[1]P. Nahinsky and S. Ruben, J. Am. Chem Soc., *63*, 2275 (1941).
[2]P. Nahinsky, C. N. Rice, S. Ruben and M. D. Kamen, *ibid.*, *64*, 2299 (1942).
[3]H. R. Mahler and A. Roberts, *ibid*, *72*, 5095 (1950).
[4]C. A. Bunton and D. R. Llewellyn, Research, *5*, 443 (1952).
[5]D. M. Hughes and J. C. Reid, J. Org. Chem., *14*, 516 (1949).

OXALIC-C_2^{14} ACID

METHOD I

$$HC^*OONa \xrightarrow{NaOH} (C^*OONa)_2 \xrightarrow[HOAc]{AgNO_3} (C^*OOAg)_2$$

(a) (b)

F. Kögl, J. Halberstadt and T. J. Barendregt, Rec. trav. chim., *68*, 387 (1949).

A. Procedure (Note 1)

(a) *Sodium Oxalate-C_2^{14}*. Sodium formate-C^{14} (0.75 mmole), dried under vacuum over phosphorus pentoxide, is pulverized in a glass tube with a little sodium hydroxide and mixed with a glass rod. The tube is evacuated to 10 μ, closed with a stopcock (Note 2) and immersed (about 1 cm.) in a metal bath at 270°. The mixture becomes molten in a few seconds, and the tube then is immersed in a bath at 440° for 2–3 minutes. Within one-half minute, the mixture froths vigorously and then sets to a hard, porous mass. The mixture is cooled, the vacuum is broken, and the mixture is taken up in 15 ml. of water. A trivial brown turbidity is removed by filtration, and the filtrate is concentrated to 10 ml. under reduced pressure over potassium hydroxide; yield 90%.

(b) *Silver Oxalate-C_2^{14}*. To a warm solution of the sodium oxalate-C_2^{14}, acidified with dilute acetic acid, is added 10 ml. of 1.5% silver nitrate

solution. The fine crystalline precipitate is collected and washed with water (Note 3).

METHOD II

$$C^*O_2 \xrightarrow[\text{sand}]{K} (C^*OOK)_2 \xrightarrow{HCl} (C^*OOH)_2$$

K. Bernhard, G. Brubacher and H. Jaquet, Helv. Chem. Acta, 36, 1968 (1953).

A. Procedure (Note 4)

To a 500-ml. round-bottomed flask containing 50 g. of fine sand heated at 150° is quickly added 4 g. of purified potassium and the flask is vigorously shaken to form a gray pasty mass. The flask is attached to a carbon dioxide generator, and the system is evacuated (30 μ). Carbon-C^{14} dioxide is generated from 3.381 g. of barium carbonate-C^{14}, and the heating is continued. The absorption of carbon dioxide is active at 190°, but moderated by slow heating to 240°, then renewed by 260° and becomes quite rapid at 270° (Note 5). The flask is stored in a moist atmosphere for 8 days; then the contents are decomposed with 10 ml. of water, and the oxalate is dissolved in 100 ml. of hot water. The solution is acidified with 60 ml. of 2 *N* hydrochloric acid, and the unreacted carbon-C^{14} dioxide is trapped in sodium hydroxide. The reaction mixture is decolorized with carbon, and the calcium salt is precipitated by adding acetate and calcium chloride. The calcium oxalate-C_2^{14} is collected and dissolved in 20 ml. of 20% hydrochloric acid, and the crude product is isolated by continuous ether extraction for 52 hours; yield 0.7248 g. Purification is accomplished by vacuum sublimation at 90–130° (50 μ), giving 0.490 g. (63%) of anhydrous oxalic-C_2^{14} acid (Note 6).

B. Notes

1. The procedure is based on the method of Matignon and Marchal.[1] A study of the reaction is presented by Leslie and Carpenter.[2]

2. A diagram of the apparatus is presented by Kögl.

3. The free acid may be obtained by treatment with hydrogen sulfide in water and evaporation of the filtered solution.

4. The procedure is a modification of that of Lermarchands.[3,10]

5. Charring begins with a further temperature rise.

6. Oxalic acid dihydrate melts at 101–102°, while the anhydrous (hygroscopic) acid melts at 189.5° (dec.). Further purification is accomplished by chromatography[4] on a column of Dowex-1 (formate form) and elution with formic acid solutions of increasing concentrations, then with 6 *N* hydrochloric-6 *N* formic acid mixtures: 1:9, 2:8 and 3:7.

C. Other Preparations

Sodium oxalate-C_2^{14} has been prepared by Weinhouse,[5] and in 88–90% yield by Murray, Bills and Ronzio,[6] essentially by Method I.

Oxalic-C_2^{14} acid has been prepared similarly by Anker[7,2] (51%).

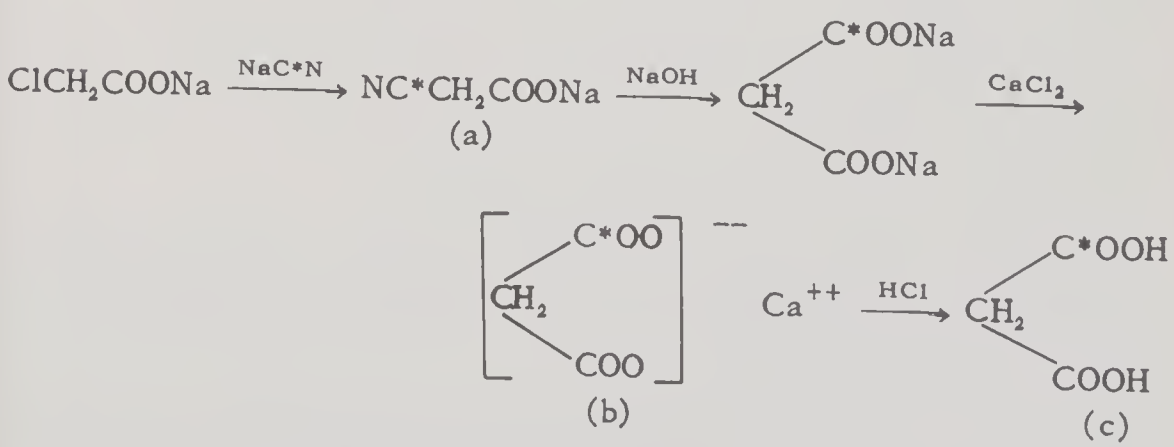
Silver oxalate-C_2^{14} has been prepared in 65.2% yield based on barium carbonate-C^{14} by Stumpf,[8] employing Method I.

Oxalic-C_2^{14} acid has been prepared by Fry[9] in 45–55% yield, corrected for 15–20% recovery of starting material, by heating carbon-C^{14} dioxide with potassium on sand at 360°. Curtin[11] has reported the preparation by fusion of potassium carbonate-C^{14} and potassium in the presence of sand. The product was shown to be radioactively homogeneous by chromatography and radioautography.

Potassium oxalate-C_2^{11} has been prepared by Long[10] (50% yield) by reacting carbon-C^{11} dioxide with molten potassium.

[1]C. Matignon and G. Marchal, Bull. soc. chim. France, Mem. (4) *31*, 789 (1922); W. Wallace, Can. 213, 377; Chem. Abstracts, *16*, 1110 (1922).
[2]E. H. Leslie and C. D. Carpenter, Chem. Met. Eng., *22*, 1195 (1920).
[3]M. Lemarchands and H. L. Roman, Compt. rend., *192*, 1381 (1931).
[4]H. Busch, R. B. Hurlbert and V. R. Potter, J. Biol. Chem., *196*, 717 (1952).
[5]S. Weinhouse and B. Friedmann, J. Biol. Chem., *191*, 707 (1951).
[6]A. Murray III, C. W. Bills and A. R. Ronzio, J. Am. Chem. Soc., *74*, 2405 (1952).
[7]R. M. Anker and J. W. Boehne, III, J. Am. Chem. Soc., *74*, 2431 (1952).
[8]W. Stumpf, F. Weygand and O. A. Grosskinsky, Ber., *86*, 1389 (1953).
[9]A. J. Fry, Nucleonics, 7 (3), 59 (1950); A. Fry and M. Calvin, J. Chem. Phys., *56*, 897 (1952).
[10]F. A. Long J. Am. Chem. Soc., *61*, 570 (1939).
[11]C. O. H. Curtin and C. G. King, J. Biol. Chem., *216*, 539 (1955).

MALONIC-1-C^{14} ACID



M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 191.

A. Procedure (Note 1)

(a) *Sodium Cyano-C^{14}-acetate.* To a stirred solution of 106 mmoles of sodium chloroacetate in 15 ml. of water is added an alkaline solution of 6 g. (123 mmoles) of sodium cyanide-C^{14} in 15 ml. of water with cooling as necessary (Note 2). When the exothermic reaction is over, the mixture is heated on a steam-bath for 40 minutes.

(b) *Calcium Malonate-1-C^{14}.* In the cooled solution of sodium cyano-C^{14}-acetate is slowly dissolved 5 g. of sodium hydroxide. The solution is heated on the steam-bath for 1.5 hours; then the last traces of evolved ammonia are removed by bubbling steam through the hot solution for 10 minutes. To the hot stirred solution of sodium malonate-1-C^{14} is added slowly 8 g. of anhydrous calcium chloride in 30 ml. of water at 50°. The mixture is stored at room temperature for 20 hours (Note 3), then filtered, and the product is washed with several 10-ml. portions of ice-water and dried (Note 4).

(c) *Malonic-1-C^{14} Acid.* To a paste prepared from the calcium malonate-1-C^{14} and 20 ml. of ether is added 12 *N* hydrochloric acid (1 ml. per 1 g. of salt) dropwise with ice-bath cooling. The resulting solution is extracted continuously with ether for at least 12 hours (Note 5). Evaporation of the extract to dryness gives the crude product; yield 8.4 g. (76% based on chloroacetate), m.p. 132° (Note 6).

B. Notes

1. The procedure is essentially that of Weiner;[1] also see malonic-2-C^{14} acid.

2. If the reaction becomes too vigorous, hydrogen cyanide is liberated and partly changed to a brown material with formation of a corresponding amount of glycolate.

3. The cheese-like precipitate becomes coarsely crystalline.

4. The dihydrate may be dried under vacuum over phosphorus pentoxide for 12 hours at 100°.

5. One type of extractor is described by Weiner.[1]

6. The acid may be recrystallized from ether-petroleum ether, or acetone-benzene, then sublimed at 90–100° (less than 1 mm.). The product is decarboxylated by heating above the melting point, forming acetic-1-C^{14} acid containing slightly more than half of the isotope due to intramolecular isotope effect.[2,3]

Purification may be accomplished also by chromatography on a silicic acid column, using 1-butanol-chloroform. The degradation of C^{14}-malonic acid is described by Yankwich and Promislow.[3]

C. Other Preparations

The preparation of calcium malonate-2-C^{14} and malonic-2-C^{14} acid by this procedure has been described in detail by Kratzl;[4] yield 104% and 87%, respectively, based on 5.2 mmoles of ester.

Calcium malonate-1-C^{14} has been prepared similarly by Gidez;[5] yield 81% based on sodium cyanide-C^{14}, or 85% based on sodium bromoacetate-C^{14}.

Sodium cyanoacetate-2-C^{14} has been prepared by Gianetto,[6] employing bromoacetate-2-C^{14}.

Yankwich[3] has described in detail a similar preparation of malonic-1-C^{14} acid; yield 70% at the 100-mmole level, m.p. 134–135°. The hydrolysis was effected by heating the nitrile with concentrated hydrochloric acid at 100° in a bomb-tube for 3 hours. The reaction mixture was evaporated at 70° under reduced pressure, and the product was extracted with ether. Malonic-1-C^{13} acid was prepared in the same manner.

[1] *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 376.
[2] G. A. Ropp and V. F. Raaen, J. Am. Chem. Soc., *74*, 4992 (1952).
[3] P. E. Yankwich and E. C. Stivers, J. Chem. Phys., *21*, 61 (1953); P. E. Yankwich and A. L. Promislow, J. Am. Chem. Soc., *76*, 4648 (1954); *75*, 4881 (1953). P. E. Yankwich, A. L. Promislow and R. F. Nystrom, J. Am. Chem. Soc., *76*, 5893 (1954); P. E. Yankwich and R. L. Belford, J. Am. Chem. Soc., *76*, 3067 (1954); A. L. Promislow, Univ. Microfilms (Ann Arbor, Mich.), Publ. No. 11, 527; Chem. Abstracts, *49*, 11334 (1955).
[4] K. Kratzl and G. Billek, Monatsh., *84*, 406 (1953).
[5] I. Gidez and M. L. Karnovsky, J. Am. Chem. Soc., *74*, 2413 (1952).
[6] R. Gianetto and L. P. Bouthillier, Can. J. Biochem. and Physiol., *32*, 154 (1954); H. A. Scarborough, Proc. Chem. Soc., *30*, 306 (1944).

MALONIC-2-C^{14} ACID

$$ClC^*H_2COOH \xrightarrow{KCN} \underset{(a)}{NCC^*H_2COOH} \xrightarrow{HCl} \underset{(b)}{C^*H_2(COOH)_2}$$

E. M. Gal and A. T. Shulgin, J. Am. Chem. Soc., 73, 2938 (1951).

A. Procedure (Note 1)

(a) *Cyanoacetic-2-C^{14} Acid.* A stirred solution of 4.1 g. of chloroacetic-2-C^{14} acid in 8 ml. of water is adjusted to pH 8 with 2.2 g. of sodium carbonate, then treated with 4.2 g. of potassium cyanide in small portions with cooling, keeping the temperature below 90° (Note 2). When the exothermic reaction has subsided, the mixture is heated on a steam-bath for 1 hour; then the solution of cyanoacetate-2-C^{14} salts is evaporated to dryness under reduced pressure. The residue is brought to pH 1.0 with con-

centrated hydrochloric acid, and the solution again is evaporated to dryness under reduced pressure (Note 3).

(b) *Malonic-2-C^{14} Acid* (Note 4). A mixture of the crude cyanoacetic-2-C^{14} acid and 30 ml. of concentrated hydrochloric acid is heated for 2 hours in a pressure stopcock-sealed tube in a boiling water-bath (Note 5). The solution is evaporated to dryness at 70° under reduced pressure in the flask of a distillation apparatus. The residue is dried for 6 hours under vacuum with warming in a water-bath (Note 6). The residue is extracted with three 50-ml. portions of absolute ether, and the solvent is evaporated, leaving the colorless crystalline product; yield 3.8 g. (84% based on chloroacetic acid), m.p. 130° (Note 7). The molar specific activity is the same as that of the starting material.

B. Notes

1. The procedure is adapted from the method of Weiner; [1] see malonic-1-C^{14} acid.

2. If the reaction becomes too vigorous, hydrogen cyanide is liberated and converted to a brown material, while a corresponding amount of glycolate is formed.

3. Pure cyanoacetic acid exists in the form of hygroscopic crystals, melting at 66°.

4. The procedure is adapted from the method of Henry.[2]

5. A water-jacketed glass bomb tube is described in the preparation of methyl-C^{14} iodide, Method I.

6. Ropp and Raaen[3] have sublimed malonic-2-C^{14} acid at 90–100° at a pressure of less than 1 mm.

7. The yield in the hydrolytic step is 93%.

C. Other Preparations

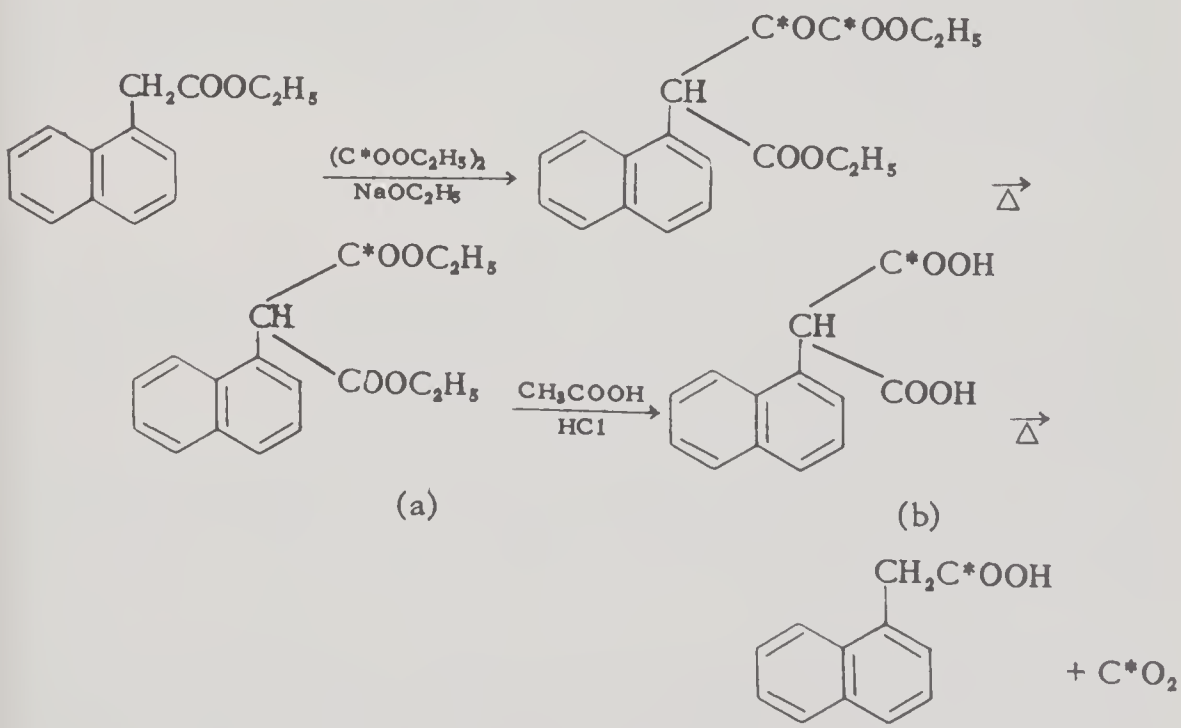
Malonic-2-C^{14} acid has been prepared in a similar manner by several investigators and purified, usually, through the calcium salt.[1] Chin,[4] Ropp[3] and Miller[5] have employed the chloroacetate-2-C^{14}; Ropp,[6] Freudenberg[7] and Gidez[8] have used the bromoacetate-2-C^{14}; and all have hydrolyzed the cyanoacetate-2-C^{14} in basic medium according to the method of Weiner.[1] Gidez[8] has reported an over-all yield of 85% based on bromoacetate. Ropp[6] has reported a yield of 76% based on potassium acetate, determined by assaying a solution of sodium malonate-2-C^{14} by isotope dilution and conversion to 2-furanacrylic-α-C^{14} acid.[9] The molar specific activity of the latter acid agreed with the calculated value based on bromoacetic-2-C^{14} acid, thus indicating that there was no isotopic rearrangement. The free malonic-2-C^{14} acid was isolated by continuous ether extraction for 66 hours in 64% yield (corrected for 86% purity) based on potassium acetate. The product was halogen-free and shown to be free of

cyanoacetic-2-C^{14} acid, by dilution with carrier cyanoacetic acid and conversion to nonisotopic α-cyanocinnamic acid.[10]

Malonic-2-C^{14} acid has been recovered by Bevington[11] from the ethyl ester by hydrolysis for 5 hours with a slight excess of boiling alcoholic potassium hydroxide. The potassium salt was collected, and the acid was liberated with hydrochloric acid, collected by ether extraction and purified by recrystallization from ether-petroleum ether. Kraml[12] has performed the saponification with aqueous calcium hydroxide under reflux and the extraction at 0.5°.

[1]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 376.
[2]Henry, Compt. rend., *102*, 1396 (1886).
[3]G. A. Ropp and V. F. Raaen, J. Am. Chem. Soc., *74*, 4992 (1952).
[4]D. Chin and P. Adams, Atomic Energy Commission Report, UCRL-2394; Nuc. Sci. Abstracts, *8*, 1057 (1954).
[5]C. S. Miller, S. Gurin and D. W. Wilson, J. Am. Chem. Soc., *74*, 2892 (1952).
[6]G. A. Ropp, J. Am. Chem. Soc., *72*, 4459 (1950); Nucleonics, 7 (3), 58 (1950).
[7]K. Freudenberg and W. Fuchs, Ber., *87*, 1824 (1954).
[8]L. I. Gidez and M. L. Karnovsky, J. Am. Chem. Soc., *74*, 2413 (1952).
[9]*Organic Syntheses*, Vol. 25, Wiley, New York, 1945, p. 51.
[10]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 181.
[11]J. C. Bevington, G. M. Guzman and H. W. Melville, Proc. Roy. Soc. (London), *221A*, 453 (1954).
[12]M. Kraml and L. P. Bouthillier, Can. J. Biochem. and Physiol., *33*, 590 (1955).

1-NAPHTHALENEMALONIC-C_1^{14} ACID



A. Fry and M. Calvin, J. Phys. Chem., *56*, 901 (1952).

A. Procedure

(a) *Ethyl 1-Naphthalenemalonate-C_1^{14}*. Ethyl oxalate-C_2^{14} is condensed with ethyl 1-naphthaleneacetate according to the procedure of Blicke,[1] which follows.

To a stirred solution of 11.5 g. of sodium in 250 ml. of absolute ethanol is added 73 g. of ethyl oxalate followed by 112 g. of ethyl 1-naphthaleneacetate. Heating is applied to the start of refluxing, then discontinued. The solidified mixture (15 minutes) is collected on a filter and washed with ether. The solid (158 g.) is distributed between 700 ml. of water and 200 ml. of ether, with stirring, and the aqueous layer is made just acidic to litmus with 50% sulfuric acid (60 ml.). The aqueous layer is separated and extracted with ether. The combined ether solution is dried over magnesium sulfate and concentrated in a Claisen flask.

The residual ethyl α-ethoxalyl-C_2^{14}-1-naphthaleneacetate is mixed with 20 g. of powdered glass and heated at 175° (15 mm.) until no further carbon-C^{14} monoxide is evolved (1 hour). The product is distilled; yield 104 g. (69%), b.p. 178–182° (2–3 mm.). The melting point is 62° after recrystallization from petroleum ether (60–70°).

(b) *1-Naphthalenemalonic-C_1^{14} Acid* (Note 2). A solution of 5.26 g. of ethyl 1-naphthalenemalonate-C_1^{14}, 25 ml. of glacial acetic acid and 2 ml. of concentrated hydrochloric acid is stored for 6 weeks. The solution is evaporated in an air stream, and the residue is triturated with benzene and filtered (Note 3); yield 2.92 g. (69.4%), m.p. 156–162° (dec.). Purification is accomplished by dissolution in alkali and washing of the solution with ether, then decolorization with carbon, acidification and extraction into ether solution. The extract is concentrated and filtered, then diluted with a large excess of benzene and stored to crystallize the product; m.p. 162–165° (dec.) (Note 4).

B. Notes

1. Directions are given by Blicke[1] for preparing ethyl 1-naphthaleneacetate (b.p. 180–181° at 15 mm.) by condensing 1-(chloromethyl)naphthalene with potassium cyanide, followed by acid hydrolysis and esterification of the 1-naphthaleneacetic acid (m.p. 131–132°).

2. The most satisfactory hydrolysis procedure is that of transesterification. The carbon dioxide evolved upon acidification, following alkaline hydrolysis of the ester, is not due to decarboxylation of the malonic acid but rather to basic cleavage of the ester, forming 1-naphthaleneacetic acid.

3. The filtrate contains mono- and diesters and any 1-naphthaleneacetic-C^{14} acid (m.p. 128–130°).

4. The decarboxylation at 163.0° takes place with 7.6 ± 0.5% isotope effect.

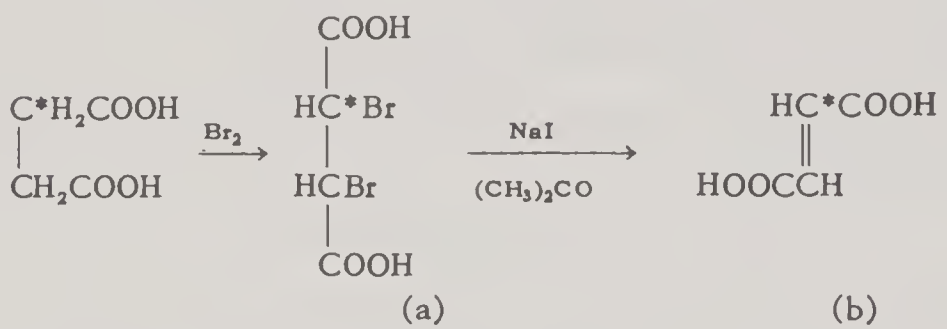
C. Other Preparations

By a similar procedure from ethyl phenylacetate and ethyl oxalate-C_2^{14}, Fry and Calvin have prepared: ethyl ethoxalyl-C_2^{14}-phenylacetate, ethyl phenylmalonate-C_1^{14} and phenylmalonic-C_1^{14} acid (m.p. 162–165°, dec.). The decarboxylation of the acid at 163.0° takes place with 8.8 ± 1.8% isotope effect.

[1]F. F. Blicke and R. F. Feldkamp, J. Am. Chem. Soc., 66, 1087 (1944).

FUMARIC-2-C^{14} ACID

METHOD I



R. F. Nystrom, Y. H. Loo and J. C. Leak, J. Am. Chem. Soc., 74, 3434 (1952).

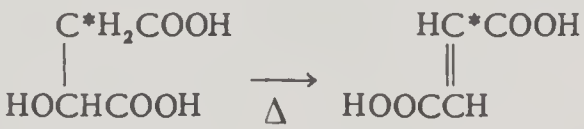
A. Procedure

(a) meso-*2,3-Dibromosuccinic-2-*C^{14} *Acid* (Note 1). A mixture of 0.295 g. (2.5 mmoles) of succinic-2-C^{14} acid, 0.24 ml. of water, 0.06 ml. of 48% hydrobromic acid and 0.28 ml. of bromine is heated at 95–100° for 96 hours in a sealed Pyrex combustion tube (Note 2). The cooled tube is opened and placed in a desiccator over calcium hydride, and the system is evacuated to 1 mm. for 2 hours. The product is removed by dissolution in three 4-ml. portions of acetone (Note 3); yield 90–96%.

(b) *Fumaric-2-*C^{14} *Acid.* The combined acetone solutions from four preparations of *meso*-2,3-dibromosuccinic-2-C^{14} acid are refluxed for 75 minutes with a solution of 4.5 g. (30 mmoles) of sodium iodide in 20 ml. of acetone. The mixture is cooled to room temperature, and the liberated iodine is titrated with *N* sodium thiosulfate. The solution is acidified with 5 ml. of glacial acetic acid and extracted continuously with ether for 24 hours. The extract is evaporated to dryness in an air stream on a steam-bath; then the residue is extracted with boiling water, and the aqueous solution is acidified with 5 ml. of glacial acetic acid and extracted continuously with ether for 24 hours. The extract is evaporated to dryness, and the crude product is dried under vacuum over calcium hydride. Purification is effected by dissolution in 50 ml. of boiling water, treatment with 50 mg. of Darco S-51, heating to boiling

after 20 minutes and filtering, then concentrating the filtrate to 9 ml. and storing at 0° for 8 hours. The crystalline product is collected on a sintered glass funnel and dried as before; yield 0.935 g., m.p. 268° (Note 4). A second crop of 0.060 g., obtained by concentrating the mother liquor to 2 ml., raises the yield to 86% based on succinic acid (Note 5). The preparation of a paper chromatogram and radioautograph (Note 6), a semi-micro quantative hydrogenation, and the preparation[1] of methyl fumarate-2-C^{14}, m.p. 104°, all indicate homogeneity of the product.

METHOD II



E. C. Jorgensen, J. A. Bassham, M. Calvin and B. M. Tolbert, J. Am. Chem. Soc., 74, 2418 (1952).

A. Procedure (Note 7)

Malic-3-C^{14} acid (0.150 g., 1 mmole) is heated for 2.5 hours at 160-170° in a 2-ml. sealed tube. The product is crystallized from 0.5 ml. of hot water in a 1-ml. centrifuge tube, collected and washed with a little cold water. The combined supernatant liquid and washes are evaporated to dryness, and the residue is reheated in a sealed tube; the process is repeated three times, making the yield 0.105 g., 81% (Note 8). Chromatography and radioautography[2] do not indicate the presence of any impurities (Note 9).

B. Notes

1. The procedure is a modification of the method of Hughes and Watson,[3] who have studied the reaction extensively.

2. The tube (15 × 20 × 250 mm.) is placed in a steel jacket. Unsafe pressures develop when a 10-mmole scale bromination is run in a single tube.

3. *meso*-2,3-Dibromosuccinic acid sublimes above 250°.

4. The melting point was obtained using polarized light and the Kofler micro melting point technique. The melting point is 287° in a sealed capillary.

5. The yield is 76% at the 10-mmole scale, and 63% at the 2-mmole scale, based on carbon dioxide.

6. The chromatogram is developed in a solvent system of phenol-water-90% formic acid (70:30:1) according to the procedure of Stark.[4]

7. The procedure is that of Wislicenus.[5]

8. Pure fumaric acid melts at 286–287°, in a sealed capillary with rapid heating; sublimation occurs at 200°.

9. The formation of maleic acid and maleic anhydride has been reported[5] under these conditions. Tests show that these likely impurities would separate readily with the solvents used and would not be lost by volatilization from the paper.

Allen and Ruben[6] have reported that the formic acid formed by the oxidative degradation of fumaric acid, with acid permanganate, is derived exclusively from the methine carbons.

C. Other Preparations

Fumaric-1-C^{14} acid is prepared[2] from malic-4-C^{14} acid by Method II.

[1]J. DeWolf and L. Van de Straete, Bull. soc. chim. Belg., *44*, 288 (1935).
[2]A. A. Benson, J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas and W. Stepka, J. Am. Chem. Soc., *72*, 1710 (1950).
[3]E. D. Hughes and H. B. Watson, J. Chem. Soc., *1930*, 1733.
[4]J. B. Stark, A. E. Goodban and H. S. Owens, Anal. Chem., *23*, 413 (1951).
[5]J. Wislicenus, Ann., *246*, 91 (1888).
[6]M. B. Allen and S. Ruben, J. Am. Chem. Soc., *64*, 948 (1942).

ACETYLENEDICARBOXYLIC-C_2^{14} ACID

$$CH_2{=}CHBr \xrightarrow{RLi} CHLi{=}CHBr \rightarrow CH{\equiv}CH \xrightarrow{RLi}$$
$$LiC{\equiv}CLi \xrightarrow{C^*O_2} HOOC^*C{\equiv}CC^*OOH$$

Y. J. Topper, J. Biol. Chem., *177*, 303 (1949).

A. Procedure (Note 1)

To 0.10 mole of butyllithium (Note 2) in 200 ml. of ether at 0° is added a solution of 3.2 g. (30 mmoles) of vinyl bromide (Note 3) in 30 ml. of absolute ether at 0°, and the mixture is stirred for 15 minutes. An aliquot (10 ml.) of the milky white dilithium acetylide solution is added to 0.33 mmole of carbon-C^{14} dioxide generated from 0.100 g. of barium carbonate-C^{14}. The mixture is stored at −35° for 24 hours, then treated with 1.5 g. of Dry Ice. When the solid carbon dioxide has disappeared, the mixture is treated with 1.0 g. of carrier acetylenedicarboxylic acid and 10 ml. of 10% sulfuric acid at 0°. The product is extracted into eight 10-ml. portions of ether, the combined extracts are concentrated, and the residue is dissolved in 5 ml. of water and treated with 2 ml. of 20% potassium hydroxide. The mixture is stored at 0° for 15 hours to crystallize the potassium hydrogen acetylenedicarboxylate-C_2^{14}. The salt is collected, washed with acetone and dissolved in 10 ml. of 10% sulfuric acid, and the free acid is extracted into ether. The ether

solution is dried over sodium sulfate, concentrated, and dried over sulfuric acid at reduced pressure; yield 0.685 g., radiochemical yield about 11% (Note 4).

B. Notes

1. The procedure is an adaptation of the method described by Gilman and Haubein.[1] Shelberg and Tolbert[2] found that the carbonation of acetylenedimagnesium bromide under various conditions was impractical.

2. See *p*-aminobenzoic-C^{14} acid, Note 4.

3. The starting material is prepared by dropping ethylene bromide into an excess of alcoholic potassium hydroxide according to the directions of Kharasch.[3]

4. Gilman and Haubein[1] have reported a yield of 34% at the 50-mmole level. Pure acetylenedicarboxylic acid dihydrate melts with decomposition at 176°. Acetylenedicarboxylic-C_2^{14} acid has been converted to fumaric-2,3-C_2^{14} acid (90% yield) by Nystrom,[4] through controlled catalytic hydrogenation in ether solution over palladium.

[1]H. Gilman and A. H. Haubein, J. Am. Chem. Soc., 67, 1420 (1945).

[2]W. E. Shelberg and B. M. Tolbert, Nucleonics, 7 (3), 56 (1950).

[3]M. S. Kharasch, M. C. McNab and F. R. Mayo, J. Am. Chem. Soc., 55, 2521 (1933).

[4]R. F. Nystrom, Y. H. Loo, K. M. Mann, J. R. Allen, Nucleonics, 7 (3), 57 (1950).

SUCCINIC-2-C^{14} ACID

METHOD I

$$ClC^*H_2COOH \xrightarrow{C_2H_5OH} ClC^*H\,COOC_2H_5 \xrightarrow[NaOC_2H_5]{CH_2(COOC_2H_5)_2}$$

(a)

$$\begin{array}{l} C^*H_2COOC_2H_5 \\ | \\ CH(COOC_2H_5)_2 \end{array} \xrightarrow{NaOH} \begin{array}{l} C^*H_2COONa \\ | \\ CH(COONa)_2 \end{array} \xrightarrow[\Delta]{H^+} \begin{array}{l} C^*H_2COOH \\ | \\ CH_2COOH \end{array}$$

(b)

E. C. Jorgensen, J. A. Bassham, M. Calvin and B. M. Tolbert, J. Am. Chem. Soc., 74, 2418 (1952).

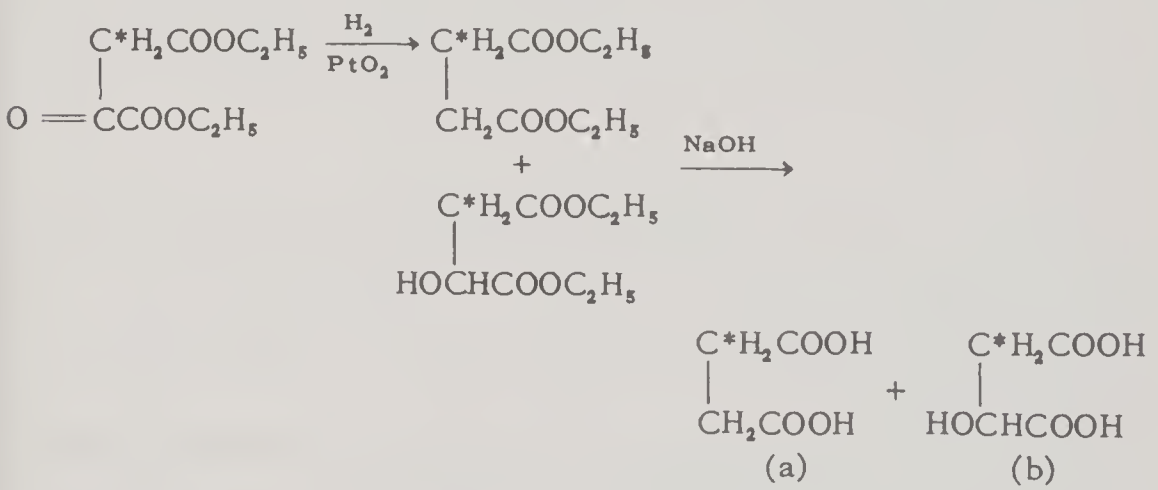
A. Procedure

(a) *Ethyl Chloroacetate-2-C^{14}* (Note 1). The crude chloroacetic-2-C^{14} acid prepared from 1.15 g. (14 mmoles) of sodium acetate-2-C^{14} is extracted continuously from 5 ml. of water with ether for 15 hours (Note 2). The extract is dried over sodium sulfate, and the ether is fractionally distilled. The chloroacetic-2-C^{14} acid is transferred with 15 ml. of

absolute ethanol into the pot of a 50-ml. Soxhlet extractor containing 5 λ of 95% sulfuric acid. The extractor cup is filled with anhydrous calcium sulfate (4 mesh), and the mixture is heated for 6 hours under dry reflux.

(b) *Succinic-2-C^{14} Acid.* To a refluxing solution of 8 g. (50 mmoles) of ethyl malonate in a solution of 1.15 g. (50 mmoles of sodium in 20 ml. of absolute ethanol is added slowly the ethyl chloro-acetate-2-C^{14} solution over a period of 1 hour (Note 3). After an additional 30 minutes of heating, the mixture is treated with 25 ml. of 4 *N* sodium hydroxide and refluxed overnight. The alcohol is distilled off, and the aqueous solution is washed into a liquid-liquid extractor, then slowly acidified with 10 ml. of 18 *N* sulfuric acid and extracted with ether for 20 hours. The ether is evaporated, and the residue is heated for 2 hours at 150–160°, the final heating being under a water-pump vacuum to decarboxylate the 2-carboxysuccinic-3-C^{14} acid and the excess malonic acid((Note 4). The crude acid is dissolved in 15 ml. of hot water and decolorized with charcoal; then the filtered solution is evaporated to crystallize the product; yield 1.47 g., m.p. 188–190°, 59.4% based on acetic-2-C^{14} acid (Note 5). The specific activity agrees with the calculated value.

METHOD II



E. C. Jorgensen, J. A. Bassham, M. Calvin and B. M. Tolbert, J. Am. Chem. Soc., 74, 2418 (1952).

A. Procedure

(a) *Succinic-2-C^{14} Acid* (Note 6). Crude ethyl oxalacetate-3-C^{14}, prepared from 17.4 mmoles of sodium acetate-2-C^{14}, is hydrogenated in 50 ml. of absolute ethanol at room temperature and ordinary pressure over 0.50 g. of platinum oxide (Note 7). After 3 hours, 0.10 g. of catalyst is added, and the reaction is continued for 2 hours to 75% reduction. The catalyst is filtered off and washed with alcohol; then the filtrate is treated with 175 ml. of 0.5 *N* sodium hydroxide, and the mixture is stirred

for 10 hours to saponify the esters. The solution is concentrated to small volume at room temperature under reduced pressure, then neutralized with cold 6 *N* sulfuric acid, and the excess oxalic acid is precipitated with 15 ml. of *M* calcium chloride. The salt is separated, and the filtrate is acidified with sulfuric acid and steam-distilled to recover unreacted sodium acetate-2-C^{14} (Note 8). The residue is concentrated to 15 ml. and extracted continuously with ether for 50 hours. The extract is evaporated, the residue is taken up in 3 ml. of 0.1 *N* hydrochloric acid, and the two product acids are separated by repeated distribution between ether and water (Note 9). The fractional extraction is continued until over 99% of the more soluble component is contained in the combined solution of each phase (Note 10). The combined ether solution is evaporated, and the residue is recrystallized from ethyl acetate-hexane. The yield of succinic-2-C^{14} acid is 24% based on recovered sodium acetate (Note 11). The purity is checked by two-dimensional paper chromatography[1] (Note 12) and radioautography.

(b) *Malic-3-C^{14} Acid.* The combined aqueous solution is evaporated to dryness, and the residue is recrystallized from ethyl acetate-hexane; yield 39% based on unrecovered sodium acetate (Note 13).

B. Notes

1. The esterification with ethyl sulfate has been described by Noller (see chloroacetic-1-C^{14} acid); yield 70–80% based on sodium acetate.

2. The residual aqueous solution contains only 0.3% of the initial activity.

3. The Soxhlet extractor is washed by refluxing absolute ethanol in the system. The wash contains considerable activity and is used to wash the dropping funnel. The system is protected from moisture with calcium sulfate.

4. Most of the resulting acetic acid distills; the evolved carbon dioxide is nonisotopic.

5. The purity of the product is checked by two-dimensional paper chromatography and radioautography; see Method II.

6. The procedure is adapted from the method of Faillebin[1] for hydrogenating aldehydes and ketones.

7. The mixture is stirred vigorously by means of a covered bar magnet.

8. There is recovered 0-14% of the starting material.

9. The distribution coefficient between ether and water is 0.15 for succinic acid and 0.016 for malic acid.

10. The degree of separation is determined accurately by paper chromatographic separation of the products and counting of the radioactive spots.

11. The yield was 5.6% in a run in which 14% of the starting material was recovered.

12. The chromatograms are developed in a solvent system of butanol-propionic acid-water (2:1:1.4) in one direction and phenol saturated with water in the other. Another method of one- and two-dimensional partition chromatographic separation of organic acids is described by Lugg[2] The degradation of succinic acid has been described by Wood,[3] Benson and Phares (see C^{14}-ethylenediamine).

13. The yield was 0.512 g., 25.8% in a run in which 14% of the starting material was recovered. The degradation of malic acid has been described by Benson.[4]

C. Other Preparations

By procedures of Method I, starting from sodium acetate-1-C^{14}, is prepared ethyl chloroacetate-1-C^{14} and succinic-1-C^{14} acid.

By the procedures of Method II, starting with ethyl oxalacetate-4-C^{14}, are prepared[5] succinic-1-C^{14} acid (28%) and malic-4-C^{14} acid (39%).

[1]M. Faillebin, Ann. chim., (10) *4*, 156 (1925).

[2]J. W. H. Lugg and B. T. Overell, Australian J. Sci. Research, Ser. A, *1*, 98, (1948); Chem. Abstracts, *43*, 6946 (1949); Nature, *160*, 87 (1947).

[3]H. G. Wood, C. H. Werkman, A. Hemingway and A. O. Nier, J. Biol. Chem., *139*, 377 (1941).

[4]A. A. Benson and J. A. Bassham, J. Am. Chem. Soc., *70*, 3939 (1948).

[5]A. A. Benson, J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas and W. Stepka, J. Am. Chem. Soc., *72*, 1710 (1950).

SUCCINIC-1,4-C_2^{13} ACID

$$BrCH_2CH_2Br \xrightarrow{KC^*N} NC^*CH_2CH_2C^*N \xrightarrow{KOH} \begin{matrix} CH_2C^*OOH \\ | \\ CH_2C^*OOH \end{matrix}$$

(a) (b)

M. Kushner and S. Weinhouse, J. Am. Chem. Soc., *71*, 3558 (1949).

A. Procedure

(a) *Succinonitrile-1,4-C_2^{13}*. Ethylene bromide is refluxed with potassium cyanide-C^{13}, according to the procedure of Vanino[1] (Note 1). Similar directions for the C^{14}-compound are presented by Diaper, Kirkwood and Marion,[2] as follows.

To a mixture of 0.616 g. (14.5 mmoles) of potassium cyanide-C^{14}, a trace of potassium iodide (Note 2), 1.0 ml. of water and 3.0 ml. of alcohol is added 1.61 g. (8.56 mmoles) of ethylene bromide. The mixture is refluxed for 75 minutes, then is diluted with 100 ml. of water and ex-

tracted with ether to remove the excess dibromide. The aqueous solution is acidified with 20 ml. of 5 *N* sulfuric acid and is stored at room temperature for 30 minutes (Note 3). The product is isolated by continuous extraction with chloroform and evaporation; yield 0.222 g. of crystalline product (Note 4).

(b) *Succinic-1,4-C_2^{13} Acid.* The crude dinitrile, without isolation, is saponified with alkali and, after removal of neutral material by ether extraction, the product is collected[3] by acidification and continuous ether extraction; yield 85–95%. Degradation, by pyrolytic decarboxylation of the barium salts *in vacuo* at 500° (1 hour), indicates that there is no isotopic rearrangement (Note 5).

B. Notes

1. Without isolation, the dinitrile is saponified with alkali, and the isotopic succinic acid is isolated in 85–95% yield.

2. The iodide serves as an intermediate compound catalyst.[4]

3. The isonitriles are readily hydrolyzed in dilute acid to primary amines and formic acid.

4. Succinonitrile melts at 54° and boils at 147° (10 mm.).

5. A complete degradation of succinic acid or anhydride by the Schmidt reaction is described by Phares; see ethylenediamine-C_2^{14}, Method I.

C. Other Preparations

By the same procedures, starting with 1,2-dibromoethane-C_2^{13} are prepared succinonitrile-2,3-C_2^{13}, and succinic-2,3-C_2^{13} acid; yield 40% based on barium carbonate. Mann and Nystrom[5] have reported an 89% yield by a modification of this procedure.

In a similar manner, starting with 1,2-dibromoethane-C_2^{14} and potassium cyanide, are prepared[6,7] succinonitrile-2,3-C_2^{14} and succinic-2,3-C_2^{14} acid.

Allen and Ruben[8] have prepared succinonitrile-1,4-C_2^{11} (50% yield) by heating an aqueous solution of potassium cyanide-C^{11} with excess ethylene chloride in a sealed tube at 150° for 15 minutes. The nitrile was quantitatively hydrolyzed to succinic-1,4-C_2^{11} acid by heating with 12 *N* hydrochloric acid for 10 minutes at 100°.

The preparation of succinonitrile-1,4-C_2^{13} and succinic-1,4-C_2^{13} acid (m.p. 183–185°), essentially by the outlined procedure, has been described by Lee and Lifson[9] Purification was achieved through the silver salt, and a degradation is described.

Succinic-1,4-C_2^{14} acid has been prepared quantitatively at the 6-mmole scale by Topper.[10] Acetylenedicarboxylic-C_2^{14} acid was hydrogenated in aqueous solution (15 ml.) at room temperature and atmospheric pressure

over 0.25 g. of 10% platinum-charcoal, the theoretical uptake being observed in 3 hours. The product was recrystallized from water, m.p. 185°, yield 11% based on barium carbonate. The preparation of succinic-1,4-C_2^{14} acid (m.p. 189–190°) by this method has been reported by Olson.[11]

[1]L. Vanino, *Handb. d. prep. Chem.*, Vol. II, 3rd Ed., F. Enke, Stuttgart, 1937, p. 263.
[2]D. G. M. Diaper, S. Kirkwood and L. Marion, Can. J. Chem., *29*, 964 (1951).
[3]S. J. Ajil and M. D. Kamen, J. Am. Chem. Soc., *73*, 2349 (1951).
[4]H. B. Hass and J. R. Marshall, Ind. Eng. Chem., *23*, 352 (1931).
[5]K. M. Mann and R. F. Nystrom, J. Am. Chem. Soc., *73*, 5894 (1951).
[6]R. F. Nystrom, Y. H. Loo, K. M. Mann and J. R. Allen, Nucleonics, *7* (3), 61, 57 (1950).
[7]S. A. Brown and A. C. Neish, Can. J. Biochem. and Physiol., *32*, 170 (1954).
[8]M. B. Allen and S. Ruben, J. Am. Chem. Soc., *64*, 948 (1942).
[9]J. S. Lee and N. Lifson, J. Biol. Chem., *193*, 253 (1951).
[10]Y. J. Topper, J. Biol. Chem., *177*, 303 (1949).
[11]R. E. Olson, O. N. Miller, Y. J. Topper and F. J. Stare, J. Biol. Chem., *175*, 503 (1948).

SUCCINIC-2,3-C_2^{14} ACID

$$\begin{matrix}CH_3COO \\ | \\ CH_3COO\end{matrix} \xrightarrow{C^*H_3COOH} \begin{matrix}C^*H_2COOH \\ | \\ C^*H_2COOH\end{matrix} + C^*H_3COOC^*H_3 + CO_2 + C^*H_4$$

A. Fry, B. M. Tolbert and M. Calvin, Atomic Energy Commission Report, UCRL-1570; Nuc. Sci. Abstracts, *6*, 854 (1952); Trans. Faraday Soc., *49*, 1444, (1953).

A. Procedure (Note 1)

The apparatus that is used consists of a pear-shaped flask equipped with a reflux condenser and a pressure-equalizing addition tube, fitted with a nitrogen inlet, delivering both gas and liquid near the bottom of the flask (for stirring). The condenser is attached, in turn, to a spiral trap at −78°, a 2 *N* sodium hydroxide bubbler, a Drierite tube, and a heated tube containing copper oxide at 700°, followed by a 2 *N* sodium hydroxide bubble trap. To 8 ml. of acetic-2-C^{14} acid, maintained at 85–95° in a slow stream of purified dry nitrogen, is added a solution of 0.454 g. (3.85 mmoles) of acetyl peroxide (Note 2) in 2 ml. of the acid over a period of 3–5 hours. The nitrogen sweep is continued for 2 hours after the cessation of gas evolution (Note 3). The unreacted acetic-2-C^{14} acid in the reaction flask is steam-distilled for recovery as the sodium salt. The residue is extracted continuously with ether for 16 hours; then the solvent is evaporated, and the crude product (0.229 g.) is recrystallized from water, m.p. 186–187° (Note 4). The molar specific activity agrees with twice that of the starting material.

B. Notes

1. For other preparative directions, see succinic-1,4-C_2^{13} acid. The procedure is essentially that described by Kharasch[1,2] for forming succinic acids and is incidental to an extensive study of the reactions of atoms and free radicals. When acetyl peroxide decomposes upon heating, it is presumed that a free methyl radical, a molecule of carbon dioxide and a free acetoxy radical are formed. The free methyl radical then captures an α-hydrogen atom from the aliphatic acid (or derivative), and the new free radical formed dimerizes;[2] yields are nearly quantitative based on the peroxide. The preparation results from a study of the reaction mechanism which, in general, confirms that proposed by Kharasch and Gladstone.[1]

2. Acetyl peroxide is prepared from acetic anhydride and sodium peroxide, according to the procedure of Kharasch;[3] the compound is recrystallized from ether at −80° and evacuated to 1 mm. for 2 hours. Immediately before use, an aliquot of the solution is analyzed by the method of Kokatnur and Jelling[4] for the iodometric determination of peroxygen in organic compounds. Caution!—all operations on the dry peroxide are carried out behind a safety shield with tongs, and the solid is put into solution as soon as possible!

3. The test for peroxide is negative.

4. The yields of by-products are: 6.30 mmoles of carbon dioxide (1.246 g. of barium carbonate) and 4.88 mmoles of methane-C^{14} (0.963 g. of barium carbonate). In the absence of peroxide, less than 1 mg. of barium carbonate is recovered from each alkaline bubbler. The Dry Ice-cooled trap contains, in addition to some acetic-2-C^{14} acid, a small amount of methyl-C^{14} acetate-2-C^{14} (b.p. 57–59°) with a molar specific activity of 10–35% of that of the starting material.

Experiment shows that there is no isotopic exchange between acetic-2-C^{14} acid and acetyl peroxide during 7 days at room temperature, followed by 6 hours at 53°, the peroxide concentration remaining constant. There is essentially no isotopic exchange when methyl acetate is heated with acetic-2-C^{14} acid for 5 hours at 100°.

Degradations of succinic acid have been discussed by Phares;[6] details of a satisfactory complete degradation of the acid or anhydride by the Schmidt reaction are given; see ethylenediamine-C_2^{14}.

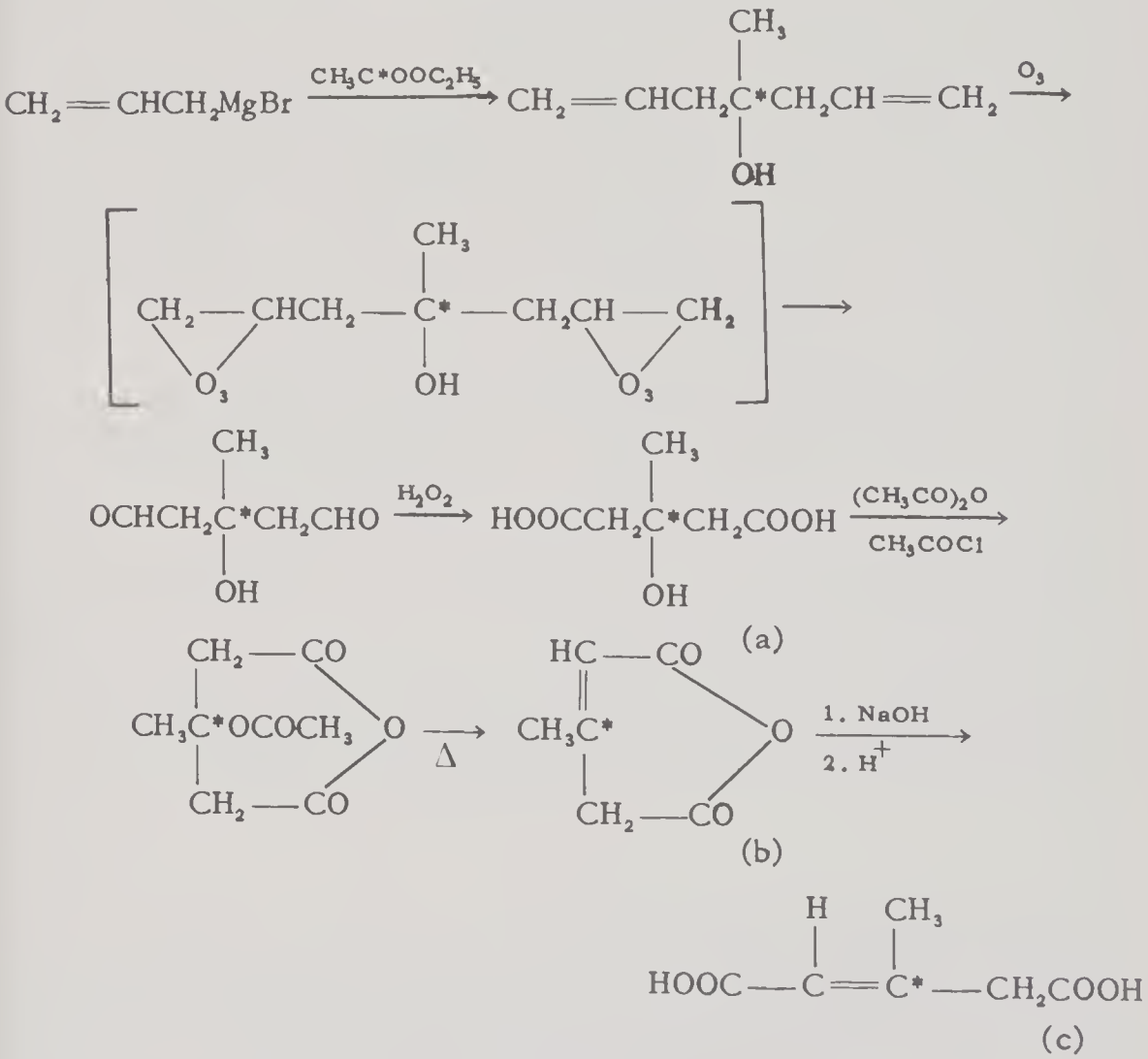
C. Other Preparations

Succinic-2,3-C_2^{14} acid has been prepared by Nystrom[5] through catalytic hydrogenation of fumaric-2,3-C_2^{14} acid, presumably in ether solution over palladium.

The preparation of succinic-2,3-C_2^{14} acid by condensation of 1,2-dibromoethane-C_2^{14} with potassium cyanide and hydrolysis of the resulting succinonitrile-2,3-C_2^{14} has been reported by Brown.[7]

[1]M. S. Kharasch and M. T. Gladstone, J. Am. Chem. Soc., *65*, 15 (1943).
[2]M. S. Kharasch, E. V. Jensen and W. H. Urry, J. Org. Chem., *10*, 386 (1945).
[3]M. S. Kharasch, H. C. McBay and W. H. Urry, J. Org. Chem., *10*, 394 (1945).
[4]V. R. Kokatnur and M. Jelling, J. Am. Chem. Soc., *63*, 1432 (1941).
[5]R. F. Nystrom, Y. H. Loo, K. M. Mann and J. R. Allen, Nucleonics, 7 (3), 57 (1950).
[6]E. F. Phares and M. V. Long, J. Am. Chem. Soc., 77, 2556 (1955).
[7]S. A. Brown and A. C. Neish, Can. J. Biochem. and Physiol., *32*, 170 (1954).

cis-3-METHYLGLUTACONIC-3-C^{14} ACID



K. Bloch, L. C. Clark and I. Harary, J. Biol. Chem., *211*, 687 (1954).

A. Procedure

(a) *3-Hydroxy-3-methylglutaric-3-C^{14} Acid* (Note 1). Ozone (Note 2) is passed through a solution of 150 ml. of ethyl acetate and 6.3 g. of 4-

methyl-1,6-heptadien-4-ol-4-C^{14}, prepared from ethyl acetate-1-C^{14} and 2 molar equivalents of allylmagnesium bromide. When the reaction is complete, the mixture is diluted with 100 ml. of glacial acetic acid and concentrated to 25 ml. under reduced pressure. The addition of acetic acid and evaporation are repeated (Note 3). A solution of the crude 3-hydroxy-3-methylglutaraldehyde-3-C^{14} in 50 ml. of glacial acetic acid is added dropwise to a stirred solution containing 25 ml. of 30% hydrogen peroxide, 50 ml. of water and 1.1 ml. of concentrated sulfuric acid (Note 4). The solution is refluxed for 2 hours; then the mineral acid is precipitated with the calculated amount of barium carbonate, and the mixture is evaporated to dryness under vacuum. The residue is extracted with acetone, the solvent is evaporated, and the crude product is recrystallized twice from acetone-benzene; m.p. 107°, radiochemical yield 14% based on ethyl acetate-1-C^{14} (Note 5).

(b) *3-Methylglutaconic-3-C^{14} Anhydride.* 3-Hydroxy-3-methylglutaric-3-C^{14} acid (0.120 g.) is converted to the unsaturated anhydride according to the procedure of Adams,[1] which follows; yield 56%, m. p. 82°.

A mixture of 0.50 g. of 3-hydroxy-3-methylglutaric acid, 10 ml. of benzene, 5 ml. of acetic anhydride and a few drops of acetyl chloride is refluxed for 2 hours, then evaporated to dryness under reduced pressure. The residual oil is triturated with ether, and the crystalline 3-acetoxy-3-methylglutaric anhydride is collected; yield 0.35 g. (Note 6).

3-Acetoxy-3-methylglutaric anhydride (0.25 g.) is decomposed to the desired product by heating at 100° for 12 hours. The resulting oil is crystallized by storage in a vacuum desiccator, and the product is purified by recrystallization from ether; m.p. 85° (Note 7).

(c) cis-*3-Methylglutaconic-3-C^{14} Acid* (Note 8). 3-Methylglutaconic-3-C^{14} anhydride is heated for 30 minutes on a steam-bath with a slight excess of 10% sodium hydroxide solution. The reaction mixture is acidified and extracted into ether solution, which is dried over sodium sulfate and concentrated to crystallize the product, m.p. 150° (Note 8).

B. Notes

1. The procedure is that of Klosterman.[2] 4-Methyl-1,6-heptadien-4-ol-4-C^{14} boils at 56–57° (14 mm.).

2. The description and procedures for use of a laboratory ozonizer are given by Smith, Greenwood and Hudrlik;[3] also see Henne.[4]

3. The spontaneous decomposition of ozonides is discussed by Henne.[4]

4. The decomposition of ozonide with hydrogen peroxide is according to the method of Henne and Hill,[5] which gives the acids in 60–70% yield.

5. The yield[1] is 81% based on 4-methyl-1,6-heptadien-4-ol-4-C^{14}.

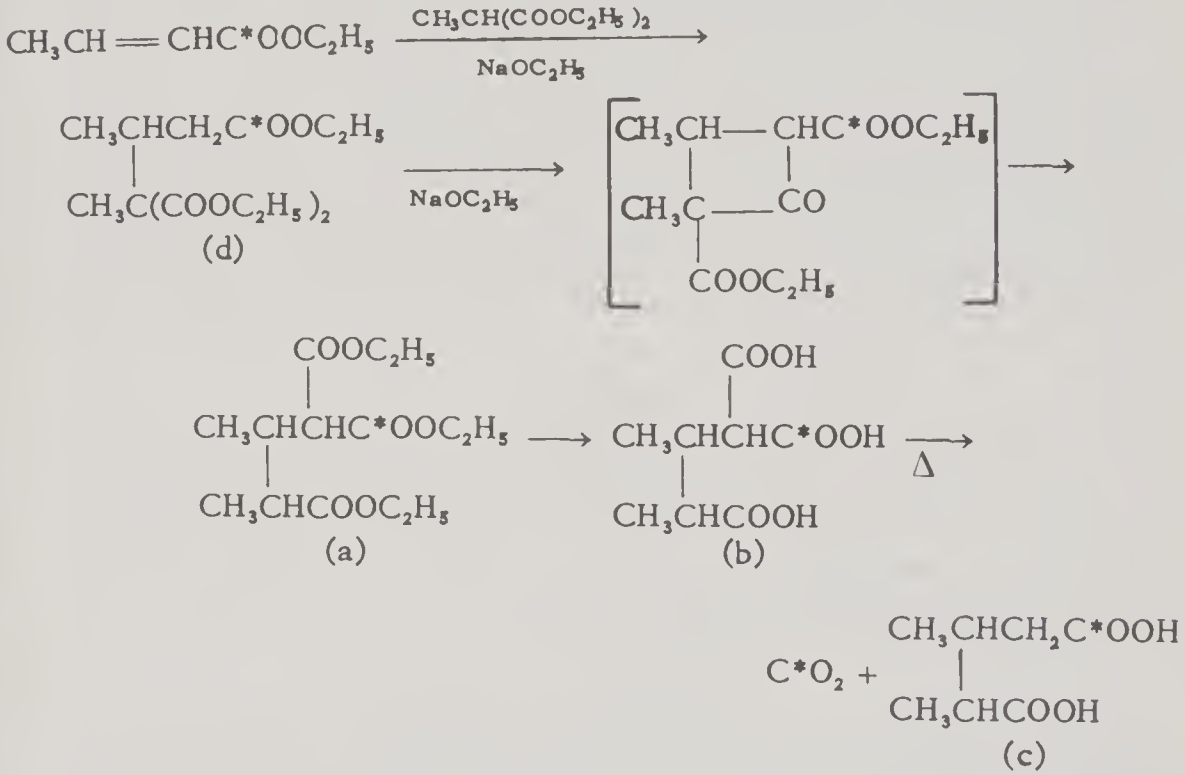
6. Recrystallization from ether gives prisms melting at 85°.

7. Directions are given by Bloch for converting the anhydride to the coenzyme A derivative.

8. The procedure is that of Adams;[1] yield 60%.

[1]R. Adams and B. L. Van Duuren, J. Am. Chem. Soc., 75, 2377 (1953).
[2]H. J. Klosterman and F. Smith, J. Am. Chem. Soc., 76, 1229 (1954).
[3]*Organic Syntheses*, Vol. 26, Wiley, New York, 1946, p. 63.
[4]A. L. Henne and W. L. Perlstein, J. Am. Chem. Soc., 65, 2183 (1943).
[5]A. L. Henne and P. Hill, J. Am. Chem. Soc., 65, 752 (1943).

2, 3-DIMETHYLGLUTARIC-5-C^{14} ACID



O. Simamura, N. Inamoto and T. Suehiro, Bull. Chem. Soc. Japan, 27, 221 (1954); through Chem. Abstracts, 49, 7494 (1955).

A. Procedure (Note 1)

(a) *Ethyl 2-Methyl-1,3-butanedicarboxylate-1-carboxylate-C^{14}.* To a mixture of 100 ml. of ether and sodium ethoxide prepared from 0.5 g. of sodium and 1.3 g. of absolute ethanol is added 3.8 g. of ethyl methylmalonate, and then dropwise 2.21 g. of ethyl crotonate-1-C^{14}. The mixture is refluxed for 5 hours, then acidified with 1.6 g. of acetic acid in 12.5 ml. of water. The ether layer is separated, dried and distilled; yield 1.7 g., b.p. 130–131° (3 mm.).

(b) *2-Methyl-1,3-butanedicarboxylic-1-carboxylic-C^{14} Acid* (Note 2). Hydrolysis of the ester gives the acid; yield 1.1 g., neutralization equiv. 73.9.

(c) *2,3-Dimethylglutaric-5-C^{14} Acid.* The tricarboxylic acid (0.17 g.) is heated gradually to 180° and then for 1 hour at 180° under vacuum, the evolved carbon-C^{14} dioxide being condensed in a trap with liquid nitrogen (Note 3).

B. Notes

1. The preparations are incidental to a study of the mechanism[1,2] of the abnormal Michael addition[3] of ethyl methylmalonate to ethyl crotonate. A similar tracer study is reported by Swan.[4] Reaction in the presence of catalytic amounts of sodium ethoxide yields the normal addition product (d), ethyl 2-methyl-3,3-butanedicarboxylate-1-carboxylate-C^{14}, reaction in the presence of 1 molecular amount of the catalyst yields the abnormal product (a), ethyl 2-methyl-1,3-butanedicarboxylate-1-carboxylate-C^{14}.

2. Swan[4] has effected a similar hydrolysis by refluxing with 10% sodium hydroxide for 10 hours. The acid is isolated by acidification, extraction with ether and recrystallization from acetone-chloroform.

3. The carbon-C^{14} dioxide is trapped in alkali and converted to barium carbonate-C^{14} (molar specific activity 30.1% of that of the tricarboxylic acid), thus demonstrating the migration of a carbethoxy group in support of the mechanism of Holden,[1] as opposed to that of Michael[2] (which would have given nonisotopic carbon dioxide). The carbonate contains 42% of the radioactivity of the ethyl crotonate-1-C^{14}.

4. Due to symmetry, either type of addition would give the same product in the absence of isotopic labeling.

C. Other Preparations

Swan[4] has described in detail the similar addition of ethyl malonate to ethyl crotonate-1-C^{14}, the same (normal addition) product being obtained regardless of the amount of sodium ethoxide used (Note 4). Decarboxylation of the 2-methyl-1,1-propanedicarboxylic-3-carboxylic-C^{14} acid (m.p. 136° dec.), obtained by hydrolysis of the ethyl 2-methyl-1,1-propanedicarboxylate-3-carboxylate-C^{14} (b.p. 145° at 1.5 mm.), gave only nonisotopic carbon dioxide and 3-methylglutaric-1-C^{14} acid, thus demonstrating that the abnormal addition mechanism does not operate in the absence of the postulated reaction driving force—the greater acid strength of (a) compared with (d).

[1]N. E. Holden and A. L. Lapworth, J. Chem. Soc., *1931*, 2368.

[2]A. Michael and J. Ross, J. Am. Chem. Soc., *52*, 4598 (1930).

[3]A. Michael, J. prakt. Chem., (2) *35*, 349 (1887); R. Connor and W. R. McClellan, J. Org. Chem., *3*, 570 (1939).

[4]G. A. Swan, J. Chem. Soc., *1955*, 1039.

CALCIUM GLYCOLATE-1-C^{14}

$$ClCH_2C^*OOH \xrightarrow[H_2O]{CaCO_3} (HOCH_2C^*OO)_2Ca$$

D. M. Hughes, R. Ostwald and B. M. Tolbert, J. Am. Chem. Soc., 74, 2434 (1952).

A. Procedure (Note 1)

A solution of 10 mmoles of crude chloroacetic-1-C^{14} acid in 20 ml. of water, under a reflux condenser, is treated slowly with 4–5 g. of calcium carbonate; then the mixture is heated for 2.5 days on a steam-bath. The reaction mixture is filtered hot, and the filtrate (without the washings) is stored in a refrigerator for at least 24 hours. The crystallized product is collected, washed with absolute alcohol and dried under vacuum. The calcium carbonate is washed thoroughly with hot water, and the filtrate is concentrated to 5–7 ml. and stored in the refrigerator to crystallize a second crop (Note 2). The first two crops are shown by two-dimensional paper chromatography and radioautography to contain only one radioactive compound (Note 3); yield 65% based on sodium acetate-1-C^{14} (Note 4).

B. Notes

1. The procedure is adapted from the method of Holzer.[1]

2. A third crop contains radioactive contaminants.

3. The chromatogram is developed in butanol-propionic acid-water in one direction, and phenol-water in the second. The method would not have detected acetic acid. The molar specific activity of the product is unchanged by recrystallization from water.

4. Sometimes the dihydrated salt crystallizes out, and sometimes the anhydrous product is obtained, depending upon which reaction starts first.

Samples of calcium glycolate-1-C^{14} (5.8 μc./mg.) and calcium glycolate-2-C^{14} (4.0 μc./mg.) have been found[2] to have undergone 13% and 22% decomposition, respectively, after storage for 48 months at room temperature in the dark. The decomposition product of self-irradiation is formic-C^{14} acid.

C. Other Preparations

By the same procedure is prepared calcium glycolate-2-C^{14} from chloroacetic-2-C^{14} acid; yield 60.5% based on sodium acetate-2-C^{14}.

Directions are given by Weinhouse[3] for the preparation of glycolic-1-C^{14} acid and glycolic-2-C^{14} acid by deamination of the appropriate glycines

with nitrous acid; radiochemical yield 30%, m.p. 77° from ether, neutralization equivalent 76 (calc. 76). Heating at 100° for 30 minutes yielded bis(glycolic-1-C^{14}) anhydride, m.p. 128–130°.

[1]A. Holzer, Ber., *16*, 2955 (1883).
[2]B. M. Tolbert, P. T. Adams, E. L. Bennett, A. M. Hughes, M. R. Kirk, R. M. Lemmon, R. M. Noller, R. Ostwald and M. Calvin, J. Am. Chem. Soc., *75*, 1867 (1953); R. M. Lemmon, Nucleonics, *11* (10), 44 (1953).
[3]S. Weinhouse and B. Friedman, J. Biol. Chem., *191*, 707 (1951); A. T. Austin, J. Chem. Soc., *1950*, 149.

LACTIC-2-C^{14} ACID

METHOD I

$$CH_3C^*H_2COOH \xrightarrow[\substack{P,\ I_2\\ CH_3CH_2COCl}]{Br_2} CH_3C^*HBrCOOH \xrightarrow{ZnO}$$
(a)

$$(CH_3C^*HOHCOO)_2Zn \rightarrow CH_3C^*HOHCOOH$$
(b) (c)

D. M. Hughes, R. Ostwald and B. M. Tolbert, J. Am. Chem. Soc., 74, 2434 (1952).

A. Procedure

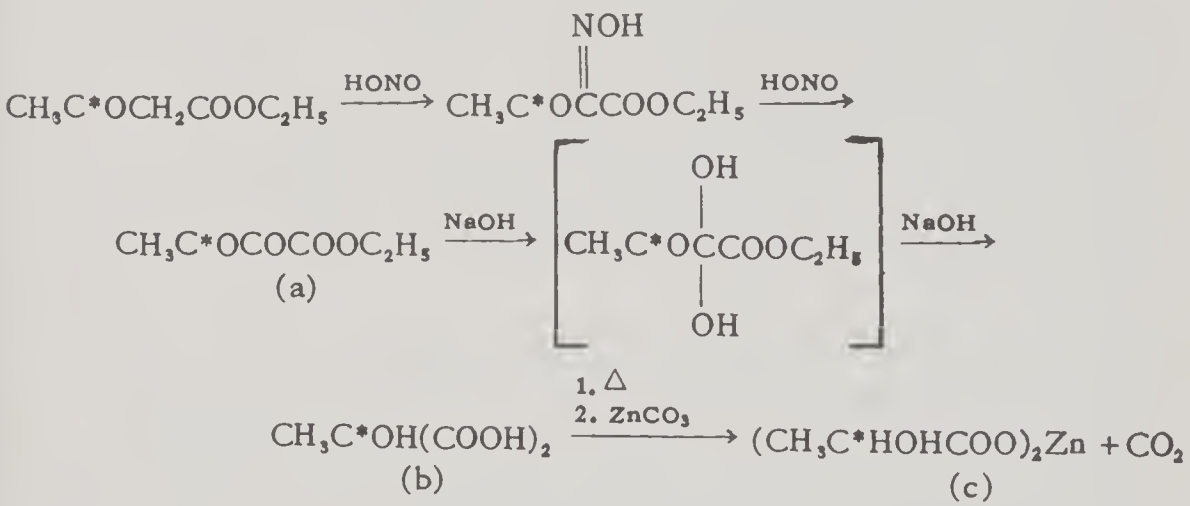
(a) *2-Bromopropionic-2-C^{14} Acid.* Dry sodium propionate-2-C^{14} (1.22 g.) is converted to the anhydrous acid by heating with hydrogen chloride (dried over phosphorus pentoxide) in a gas-solid reaction vessel (Note 1) filled to 0.5 atmospheres. The volatile acid is collected in a trap cooled with liquid nitrogen; then the trap is warmed with a Dry Ice-bath, and most of the excess hydrogen chloride is pumped off. The free acid (98–99% yield) is distilled into a semimicro bromination flask (Note 2) containing 0.04 g. of red phosphorus, 0.02 g. of iodine and 0.2 ml. of propionyl chloride. The mixture is refluxed for 30 minutes on a water-bath, to ensure anhydrous conditions; then 1.5 ml. of purified bromine is added dropwise, and heating is continued for at least 3 hours. The apparatus is closed off from the atmosphere, the mixture is frozen with liquid nitrogen, and 20 ml. of water is added slowly to hydrolyze the acid chloride, followed dropwise by *N* sodium bisulfite (15 ml.) to destroy the excess bromine.

(b) *Zinc Lactate-2-C^{14}* (Note 3). To the 2-bromopropionic-2-C^{14} acid reaction mixture is added 6 g. of zinc oxide, and the mixture is refluxed on a steam-bath for 2.5 days. The excess oxide is filtered from the hot solution and washed with 5–6 portions of hot water. The filtrate and

washings are combined, evaporated to 6 ml. and stored in a refrigerator for 2–3 days to crystallize. The crude product is collected, recrystallized from 5 ml. of 0.1 *M* zinc chloride (Note 4) and dried for 24 hours under vacuum; yield 1.325 g. The stable trihydrate of uniform hydration is formed by exposure to the atmosphere for 3 days; yield 1.624 g. (72.8%). An additional crop is obtained by recrystallizing 0.30 g. of carrier from the mother liquors and is purified by again recrystallizing; yield (anhydrous) 0.277 g., radiochemical yield 3.7%. The purity is checked by two-dimensional paper chromatography (Note 5) and radioautography.

(c) *Lactic-2-C*^{14} *Acid, (2-Hydroxypropionic-2-C*^{14} *Acid)*. An aqueous solution of the free acid is prepared quantitatively by mixing a solution of the zinc salt with excess Dowex-50 ion exchange resin, in the acid form, and filtering (Note 6). The purity is checked by two-dimensional paper chromatography and radioautography by the method described by Benson.[1]

METHOD II



H. W. Davis, E. Grovenstein, Jr., and O. K. Neville, J. Am. Chem. Soc., 75, 3304 (1953).

A. Procedure

(a) *Ethyl 2,3-Dioxobutyrate-3-C*^{14}. From 40 g. of ethyl acetoacetate-3-C^{14} is prepared 16.5 g. (37% yield) of ethyl 2,3-dioxobutyrate-3-C^{14} (b.p. 80–83° at 23 mm.) according to the procedure of Karrer[2] and Denis,[3] which follows.

To a mixture of 90 g. of ethyl acetoacetate and 60 g. of powdered sodium nitrite is added slowly a solution of 38 g. of sulfuric acid and 225 ml. of water, with cooling in an ice-salt mixture. After 2 hours, the mixture is diluted with 200 ml. of water, and the ethyl isonitrosoacetoacetate

is extracted with a total of 600 ml. of ether. The extract is dried with calcium chloride and treated at 10° for 3 hours with a rapid stream of dry nitrous acid, prepared by the action of concentrated nitric acid on arsenious oxide. The mixture is stored for 2 days, then concentrated and distilled under reduced pressure, giving a crude yield of 69.3 g., b.p. 70–85° (18 mm.). The yellow oil is dissolved in an equal weight of water and shaken in the cold with an excess of powdered calcium carbonate. The mixture is extracted with 15 portions of ether, and the extract is dried, concentrated and fractionally distilled; yield 46.5 g., b.p. 76–82° (18–22 mm.).

Refractionation with a Podbielniak column gives the pure diketone, b.p. 64–65° (9 mm.). The compound is hygroscopic, forming a crystalline monohydrate; the monophenylhydrazone melts at 102–103°.

(b) *Methyltartronic-2-C^{14} Acid*, (*Isomalic-2-C^{14} Acid*). To an ice-cooled solution of 5 g. of sodium hydroxide in 30 ml. of water is added a solution of 5 g. of ethyl 2,3-dioxobutyrate-3-C^{14} in 10 ml. of water. After a few minutes, the solution is stored for 2 days at room temperature, then is acidified with concentrated hydrochloric acid and neutralized with ammonium hydroxide. The barium salt, precipitated by treating the solution with 12 g. of barium chloride in 35 ml. of water and cooling to 0° for 30 minutes, is collected and dried in a desiccator; yield 9.1 g. (97.5%). The barium salt (5 g.) is treated with 90% of the stoichiometric amount of 1.87 *N* sulfuric acid, and the filtered solution is evaporated to dryness at 45–50° (25 mm.). The residual crude product is extracted with ether, and the filtered solution is evaporated; yield 1.4 g. (56%), m.p. 132–136°. Purification by recrystallization from benzene-ether gives 0.5 g., m.p. 142°. Degradation studies (see below) indicate that the alkaline rearrangement proceeds exclusively by migration of the carboxyl group.

(c) *Zinc Lactate-2-C^{14}*. A mixture of 0.2127 g. of methyltartronic-2-C^{14} acid and 1.0 ml. of water is heated at 80° for 48 hours, then is treated with an excess of zinc carbonate and filtered hot. The solution is diluted with 0.5 ml. of alcohol and stored at 0° for 1 hour. The precipitated zinc lactate-2-C^{14} trihydrate is collected by centrifugation, recrystallized from 1.3 ml. of 0.1 *N* zinc chloride at 0° and air-dried; yield 0.1148 g., 90%. The molar specific activity is the same as that of the starting material, and the evolved carbon dioxide is nonisotopic.

B. Notes

1. A diagram of a suitable reactor is presented by Chin and Adams.[4] Also see sodium acetate-1-C^{14}.

2. The flask consists of a 30-ml. conical flask fitted with a Dry Ice reflux condenser, attachment to a vacuum line, a Drierite-filled guard tube and a stoppered addition tube made from a 12-ml. graduated centrifuge cone.

3. The procedure is adapted from the method of Fischer.[5] Silver oxide has been used by Wood[6] for effecting the hydrolysis in an isotopic synthesis.

4. Crystallization from water results in partial hydrolysis of the salt. All crystallizations are slow.

5. The solvent system consists of butanol-propionic acid-water in one direction, and phenol-water in the second.

6. Lactic acid, isolated by continuous ether extraction,[7] may be titrated with alkali, or determined colorimetrically.[8] A degradation is described by Wood.[9] The guanidine salt has been prepared,[10] m.p. 163.5–164.5°.

C. Other Preparations

By the same procedure are prepared: zinc lactate-1-C^{14} (81.8%) and zinc lactate-3-C^{14} (76.3%), the yields being based on the appropriate labeled propionic acid at the 6 to 12-mmole scale.

Sodium lactate-2-C^{13} has been prepared in a similar manner by Lorber,[7] by hydrolyzing 2-bromopropionic-2-C^{13} acid with sodium carbonate.

The preparation of sodium lactate-2-C^{13}-3-C^{14} has been reported by Lorber.[7] Propionic-2-C^{13}-3-C^{14} acid, prepared from ethyl-1-C^{13}-2-C^{14} iodide by nitrile formation and hydrolysis, was boiled with benzoyl chloride, and the resulting propionyl-2-C^{13}-3-C^{14} chloride was distilled, brominated, and hydrolyzed with sodium carbonate. The preparation of 2-bromopropionic-1-C^{14} acid and lactic-1-C^{14} acid by this method has been reported by Rudney.[11]

Lactic-2-C^{14} acid has been prepared by Anker,[12] by hydrogenation of pyruvic-2-C^{14} acid in aqueous solution at ordinary temperature and pressure over platinum oxide.

The preparation of 2-bromopropionic-3-C^{14} acid, zinc lactate-3-C^{14} and lactic-3-C^{14} acid by essentially the described method[5,14] has been reported by Bloom.[13] Sodium lactate-3-C^{14} was formed by titrating the hot aqueous solution of free acid to pH 7.4 with sodium hydroxide. Potassium lactate-3-C^{14} has been prepared[13] by neutralizing the free acid with potassium bicarbonate.

[1]A. A. Benson, J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas and W. Stepka, J. Am. Chem. Soc., *72*, 1710 (1950).

[2]P. Karrer and E. B. Hershberg, Helv. Chim. Acta, *17*, 1020 (1934).

[3]W. Denis, Am. Chem. J., *38*, 587 (1907).

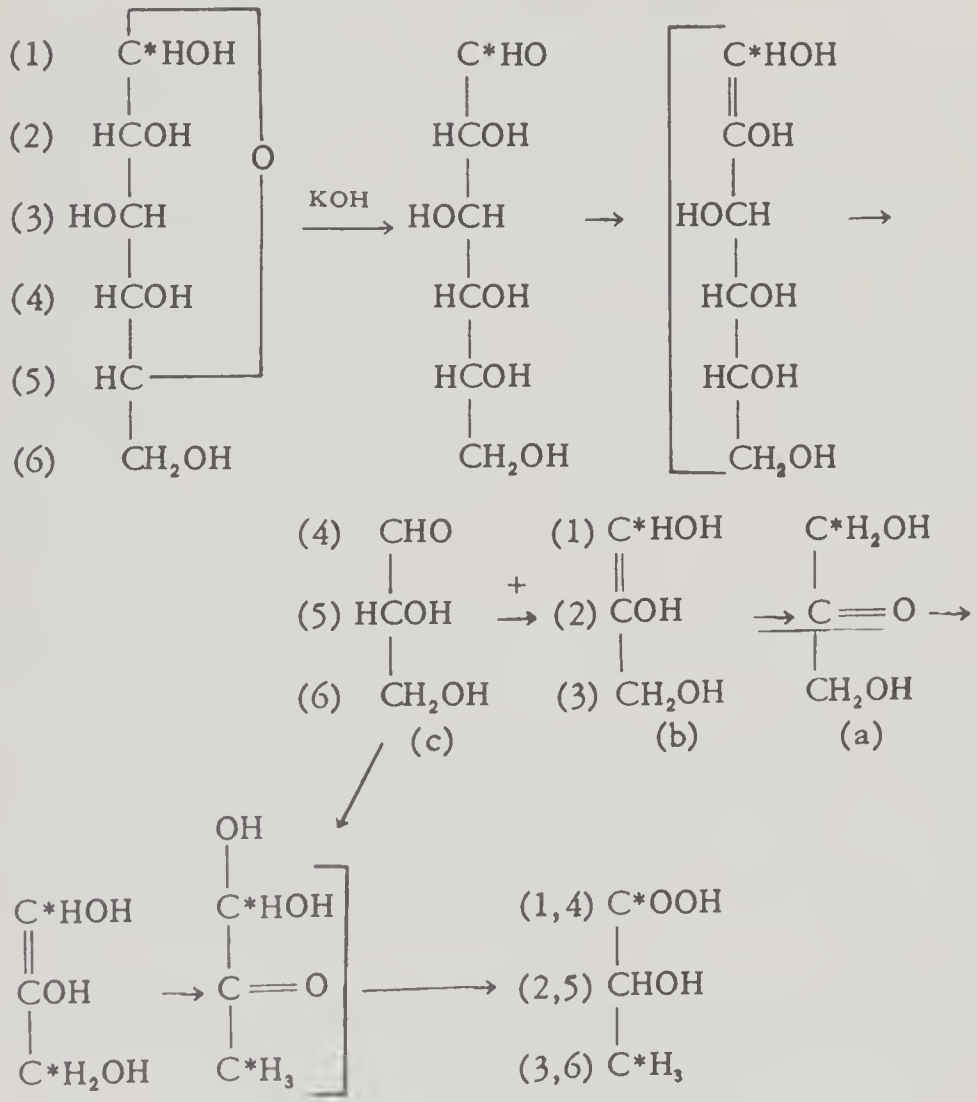
[4]D. Chin and P. Adams, Atomic Energy Commission Report, UCRL-2394; Nuc. Sci. Abstracts, *8*, 1057 (1954); D. M. Hughes and B. M. Tolbert, Atomic Energy Commission Report, AECU-121.

[5]E. Fischer and G. Zemplen, Ber., *42*, 4878 (1909).

[6]H. G. Wood, C. H. Werkman, A. Hemingway and A. O. Nier, Proc. Soc. Exptl. Biol. Med., *46*, 313 (1941).

[7]V. Lorber, N. Lifson, H. G. Wood, W. Sakami and W. W. Shreeve, J. Biol. Chem., *183*, 517 (1950).
[8]S. B. Barker and W. H. Summerson, J. Biol. Chem., *138*, 535 (1941).
[9]H. G. Wood, N. Lifson and V. Lorber, J. Biol. Chem., *159*, 475 (1945).
[10]V. Lorber and M. Cook, J. Biol. Chem., *215*, 823 (1955); M. A. Packham and G. C. Butler, J. Biol. Chem., *194*, 349 (1952).
[11]H. Rudney, J. Biol. Chem., *210*, 353 (1954).
[12]H. S. Anker, J. Biol. Chem., *187*, 167 (1950).
[13]B. Bloom, M. R. Stettin and D. Stettin, Jr., J. Biol. Chem., *204*, 681 (1953); F. Eisenberg, Jr., J. Biol. Chem., *212*, 501 (1955).
[14]*Organic Syntheses*, Coll. Vol. III, Wiley, New York, 1955, p. 848.

LACTIC-1,3-$C^{14}_{1/2}$ ACID



M. Gibbs, J. Am. Chem. Soc., *72*, 3964 (1950).

A. Procedure (Note 1)

A solution of 0.100 g. of D-glucose-1-C^{14} in 2 ml. of 3 *N* potassium hydroxide, under nitrogen (Note 2) in a stoppered vial, is heated at 50 ±

1° for 48 hours. The mixture is acidified (phenol red) and continuously extracted with ether for 36 hours. The extract is treated with 5 ml. of water, the ether is evaporated, and volatile impurities are removed by steam distillation (Note 3); the aqueous solution contains 0.045 g. of product (Note 4). Degradation studies (Note 5) indicate that the isotopic distribution is C-1 (48.5%), C-2 (3%) and C-3 (48.5%) (Note 6).

When lactic-$C^{14}_{1/3}$ acid is prepared by the same procedure from D-glucose-3,4-C^{14}_2 (Note 7), the distribution of isotope is C-1 (66.7%), C-2 (5.3%) and C-3 (28%) (Note 8).

B. Notes

1. The procedure is adapted from the studies of Plunkett and Evans[1,2] on the action of aldehyde sugars in alkaline solution. The preparation is incidental to a tracer study of the reaction mechanism.

2. Air must be excluded to avoid oxidation of the strongly reducing intermediates.

3. The equimolecular amounts of formic and acetic acids formed under these conditions of temperature and alkali concentration are small,[3] for the rate of lactic acid formation considerably exceeds that of the decomposition of pyruvaldehyde. At lower temperatures and alkali concentration an additional small amount of formic acid arises from another source, believed to be the 1,2-enediol.

4. The lactic acid is determined by the method of Friedemann;[4] also see lactic-2-C^{14} acid, Note 6. Plunkett and Evans[1] report the maximum yield as nearly 1 mole of lactic acid per mole of D-glucopyranose; see Schaffer and Friedemann[5] (p. 359, Note 6). The chief by-products are saccharinic acids.[1,2,3]

5. The lactic acid is degraded by the method of Wood[6] to carbon-C^{14} dioxide and acetaldehyde-2-C^{14}, which is converted to iodoform-C^{14} and formic acid. Also see several degradations indicated by Jefferson[7], and that described in detail by Katz.[8]

6. The randomization may occur at the symmetrical dihydroxy acetone stage (a). Comparison of the radioactivity of the product and starting material indicates that 42% of the lactic acid is formed from C-1 to 3 of glucose or triose enediol (b), and 58% originates from C-4 to 6 of glucose or glyceraldehyde (c).

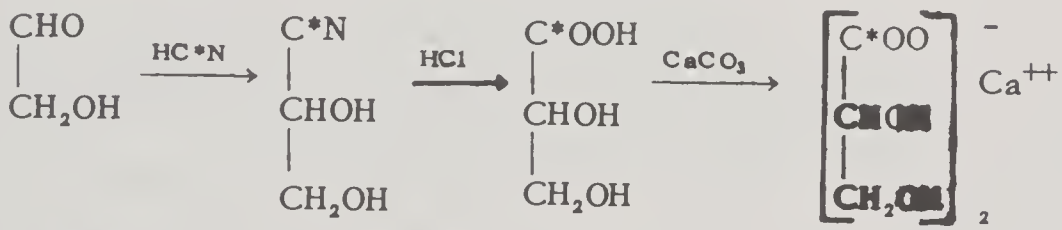
7. D-glucose-3,4-C^{14}_2 is prepared by the biosynthetic method of Topper.[9]

8. To explain a C-1/C-3 ratio of less than 3 in the lactic acid, it is probable that a partial randomization occurred between C-4 and C-6. This is not in agreement with the mechanism of Evans.[2,3]

[1]R. J. Plunkett and W. L. Evans, J. Am. Chem. Soc., *60*, 2847 (1938).
[2]W. L. Evans, Chem. Rev., *31*, 537 (1942).

[3]W. L. Evans, R. H. Edgar and G. P. Hoff, J. Am. Chem. Soc., *46*, 2665 (1926).
[4]T. E. Friedemann, J. Biol. Chem., *76*, 75 (1928).
[5]P. A. Schaffer and T. E. Friedemann, *ibid.*, *86*, 345 (1930).
[6]H. G. Wood, N. Lifson and V. Lorber, *ibid.*, *159*, 475 (1945).
[7]W. E. Jefferson, J. W. Foster, E. F. Phares and S. F. Carson, J. Am. Chem. Soc., *74*, 1477 (1952).
[8]J. Katz, S. Abraham and I. L. Chaikoff, Anal. Chem., *27*, 155 (1955).
[9]Y. J. Topper and A. B. Hastings, J. Biol. Chem., *179*, 1255 (1949).

CALCIUM GLYCERATE-1-C^{14}



H. J. Sallach, J. Am. Chem. Soc., *74*, 2415 (1952).

A. Procedure

To 10.7 mmoles of hydrogen cyanide-C^{14} (Note 1), frozen in a reaction flask containing a trace of sodium cyanide as catalyst, is added a solution of 0.700 g. (11.6 mmoles) of glycolaldehyde in 4 ml. of water (Note 2). The mixture is left at 0° for 30 minutes, then at room temperature for the same time. Concentrated hydrochloric acid (4 ml.) is added to the solution of glyceronitrile-1-C^{14}, and hydrolysis is allowed to proceed for 30 minutes. Excess calcium carbonate is added, and the mixture is boiled for 10 minutes and filtered. The calcium salt of glyceric-1-C^{14} acid is precipitated by adding 4–5 volumes of ethanol. The product is decolorized with charcoal in a minimal volume of hot water and is reprecipitated with alcohol. The yield is 33%, and the molar specific activity is the same as that of the starting material.

B. Notes

1. The hydrogen cyanide is generated in a vacuum system, by adding 10 ml. of concentrated sulfuric acid to 0.5516 g. of sodium cyanide-C^{14} and distilling the liberated acid into a flask cooled with liquid nitrogen.

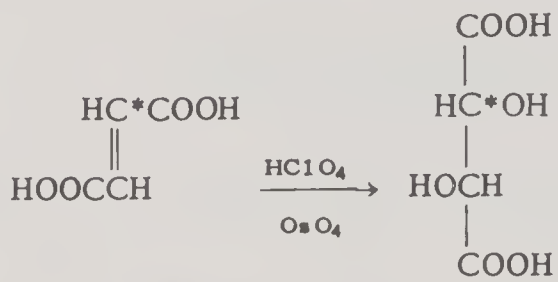
2. The solution was 24 hours old in this instance. Glycolaldehyde may be prepared by decarboxylating dihydroxymaleic acid by heating in pyridine, according to the method of Fischer and Taube.[1] The required acid may be prepared by oxidizing tartaric acid with hydrogen peroxide, according to the procedure of Fischer and Feldman.[2]

C. Other Preparations

Glyceric-1-C^{14} acid has been prepared in a similar manner by Doerschuk (see 1,2,3-propanetriol-1-C^{14}, Method I).

[1]H. O. L. Fischer and C. Taube, Ber., *60B*, 1704 (1927).
[2]H. O. L. Fischer and L. Feldman, Ber., *62B*, 854 (1929).

TARTARIC-2-C^{14} ACID



E. C. Jorgensen, J. A. Bassham, M. Calvin and B. M. Tolbert, J. Am. Chem. Soc., *74*, 2418 (1952).

A. Procedure

To a solution of 0.105 g. (0.905 mmole) of fumaric-2-C^{14} acid in 15 ml. of hot water, in a 40-ml. centrifuge tube, is added 0.143 g. (1.17 mmoles) of potassium chlorate in a few ml. of water and 0.050 ml. of freshly prepared 1% osmic acid solution (Note 1). The tube is stoppered and heated at 50° for 12 hours. The solution is cooled, extracted with benzene to remove excess osmic acid, then concentrated to 7 ml. on a steam-bath, neutralized with dilute sodium hydroxide and treated with 3 ml. of saturated barium chloride solution (Note 2). The mixture is stored overnight in a refrigerator; then the barium tartrate-2-C^{14} is collected and washed with cold water. The salt is treated with dilute sulfuric acid, and the solution is extracted continuously with ether for 18 hours. The extract is evaporated, and the crystalline product is washed with a little cold water (Note 3)[1] yield 0.0565 g. (42%). Paper chromatograms and radioautographs[1] show the presence of only one radioactive compound.

B. Notes

1. The method is that of Milas and Terry.[2]

2. The potassium acid tartrate may be isolated[3] at the 6-mmole scale by neutralizing with potassium hydroxide, concentrating to 10 ml., adding 6 meq. of glacial acetic acid and crystallizing overnight.

3. The free acid may be more readily isolated (98%) from the barium salt by use of the cation exchange resin, Dowex-50.

C. Other Preparations

By the same procedure, starting with fumaric-1-C^{14} acid, is prepared[1] tartaric-1-C^{14} acid.

Tartaric-2,3-C_2^{14} is prepared[2] from fumaric-2,3-C_2^{14}acid; yield 78%.

[1]A. A. Benson, J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas and W. Stepka, J. Am. Chem. Soc., *72*, 1710 (1950).

[2]N. A. Milas and E. M. Terry, J. Am. Chem. Soc., *47*, 1412 (1925).

[3]A. Weissbach and D. B. Sprinson, J. Biol. Chem., *203*, 1023 (1953).

MANDELIC-C^{14} ACID
(Phenylglycolic-1-C^{14} Acid)

$$C_6H_5CHO \xrightarrow[NaHSO_3]{NaC^*N} C_6H_5CHC^*N \xrightarrow{HCl} C_6H_5CHC^*OOH$$
$$\qquad\qquad\qquad\qquad\quad | \qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\quad OH \qquad\qquad\qquad\quad OH$$

C. J. Collins, J. Am. Chem. Soc., *77*, 5517 (1955).

A. Procedure

(a) *Mandelic-C^{14} Acid.* Mandelonitrile-C^{14} and the acid are prepared from sodium cyanide-C^{14} by a suitable adaptation of the procedure of Vogel,[1] which follows.

To a stirred mixture of 25 g. of sodium cyanide, 100 ml. of water and 53 g. of purified benzaldehyde (Note 1) is added a saturated solution of sodium bisulfite (250 g./335 ml. of water) during 10–15 minutes. Crushed ice (150 g.) is added in several portions during the first half of the addition. The crude mandelonitrile is separated by benzene extraction and mixed as soon as possible (Note 2) with 75 ml. of concentrated hydrochloric acid. The mixture is stored for 12 hours (Note 3) and evaporated on a steam-bath. The pulverized residue is washed with two 125-ml. portions of cold benzene (Note 4) and extracted with 200 ml. of benzene in a Soxhlet apparatus. The product crystallizes from the extract; yield 35 g., m.p. 118°.

B. Notes

1. Benzaldehyde is purified by shaking with an excess of concentrated sodium bisulfite, which is added in portions until aldehyde odor is no longer detected. The complex is collected on a filter, washed with ether and decomposed in a separatory funnel with an excess of sodium carbonate solution. The benzaldehyde is extracted into ether solution, which is washed with sodium carbonate solution and water, dried over magnesium sulfate, and distilled (b.p. 178–180°) with minimum exposure to the atmosphere.

2. Standing results in rapid conversion to the acetal of benzaldehyde and mandelonitrile and/or the isonitrile.

3. A deeply colored product is obtained if hydrolysis is carried out with heating.

4. The wash removes most of the color and a negligible amount of product.

[1]A. I. Vogel, *Practical Organic Chemistry*, Longmans, New York, 1948, p. 734; *Organic Syntheses, Coll. Vol. I, Wiley*, New York, 1941, p. 336.

MANDELIC-α-C^{14} ACID

METHOD I

$$C_6H_5C^*OCH_3 \xrightarrow{Br_2} C_6H_5C^*OCHBr_2 \xrightarrow{NaOH} C_6H_5\underset{\displaystyle OH}{\underset{|}{C^*}}HCOOH$$

(a) (b)

O. K. Neville, J. Am. Chem. Soc., *70*, 3499 (1948).

A. Procedure (Note 1)

(a) *2,2-Dibromoacetophenone-1-C^{14}.* To a strongly illuminated (photoflood lamp) solution of 1.2 g. of acetophenone-1-C^{14} and 1.5 ml. of carbon disulfide is added slowly 3.2 g. to bromine. When absorption is complete, the solution is cooled in a Dry Ice-bath to crystallize the product; yield 2.55 g. (91.8%), after recrystallization from carbon disulfide-petroleum ether.

(b) *Mandelic-α-C^{14} Acid.* A mixture of the 2,2-dibromoacetophenone-1-C^{14} and 15 ml. of 5% sodium hydroxide solution is stirred overnight at room temperature, then extracted with two portions of chloroform. The aqueous solution is neutralized with hydrochloric acid, and the product is precipitated as cadmium mandelate-α-C^{14}, which is washed with ether and dried; yield 1.81 g., 87.5%. The salt is dissolved in 20 ml. of concentrated hydrochloric acid, and the solution is diluted with a few drops of water and saturated with sodium chloride. The product is extracted into four 10-ml. portions of ether, the solvent is evaporated, and the residue is recrystallized from benzene-ether and dried under vacuum at 64°; yield 1.3 g. (85.5%), m.p. 117°. Degradation studies give results similar to those described in Method II.

METHOD II

$$C_6H_5C^*OCH_3 \xrightarrow{SeO_2} C_6H_5C^*OCHO \xrightarrow{NaOH} \underset{\displaystyle OH}{\underset{|}{C_6H_5C^*}}HCOOH$$

(a) (b)

O. K. Neville, J. Am. Chem. Soc., *70*, 3499 (1948).

A. Procedure

(a) *2-Phenylglyoxal-2-C^{14}* (Note 2). A mixture of 1.0 g. of acetophenone-1-C^{14}, 1.66 g. of selenium dioxide,[1] 6 ml. of dioxane and 0.2 ml. of water is refluxed for 8 hours. The solution is separated from the precipitated selenium with a capillary pipette, and the solvent is removed by distillation through a short column. The residual phenylglyoxal-2-C^{14} hydrate is not isolated and purified, because of losses on the small scale, but is used directly (Note 3). Degradation studies indicate that there is no isotopic rearrangement (Note 4).

(b) *Mandelic-α-C^{14} Acid.* The crude phenylglyoxal-2-C^{14} is taken up in ether and shaken for 1 hour with 30 ml. of 10% sodium hydroxide solution (Note 5). The mixture is neutralized, and the product is precipitated and purified through the cadmium salt as in Method I; yield 0.9488 g. (75.2%), m.p. 117–118°. Degradation studies indicate that there is no carbon rearrangement (Note 6).

B. Notes

1. The method is an adaptation of the method of Engler.[2]

2. The procedure is that of Riley and Gray.[1] The yield is 69–72% at the 1-mole level.

3. The phenylosazone melts at 150.6–151.4°.

4. Directions are given for oxidizing the phenylglyoxal-2-C^{14} to benzoic-C^{14} acid with periodic acid. The molar specific activity is the same as that of the acetophenone-1-C^{14} from which it is prepared in 64.4% yield.

5. The alkali is added slowly to the cooled mixture, for the reaction may be vigorous.

6. Directions are given by Neville for both the alkaline permanganate oxidation and the chromic acid oxidation of the acid to nonisotopic carbon dioxide and benzoic-C^{14} acid, with yields of 85.2 and 77.7%, respectively. The molar specific activity is the same as that of the starting material. This data invalidates several proposed reaction mechanisms,[3] including the migration of the phenyl group, as in the benzil-benzilic acid rearrangement, and supports the intramolecular migration of a hydrogen atom,[3,4] as in one of the attractive versions of the Cannizzaro reaction. Doering[3] has degraded the C^{13}-acid by both neutral and acidic permanganate oxidation followed by decarboxylation of the benzoic-C^{13} acid by the Schmidt reaction (32.2% yield).

C. Other Preparations

Mandelic-α-C^{13} acid has been prepared by Doering[3] in 24% yield (m.p. 112–114°) from 0.3 g. of benzoic-C^{13} acid, without purification of intermediates. The acid chloride was added to diazomethane,[5] and the crude

2-diazoacetophenone-1-C^{13} was brominated, forming 2,2-dibromoacetophenone-1-C^{13}, which was converted by alkali in the manner described.

Mandelic-α-C^{13} acid has been prepared in 72.5% yield by Doering,[3] by shaking 2-phenylglyoxal-2-C^{13} hydrate, prepared as above, with barium oxide and water.

[1] *Organic Syntheses*, Coll Vol. II, Wiley, New York, 1943, p. 509; H. L. Riley J. F. Morley and N. A. C. Friend, J. Chem. Soc., *1932*, 1875.

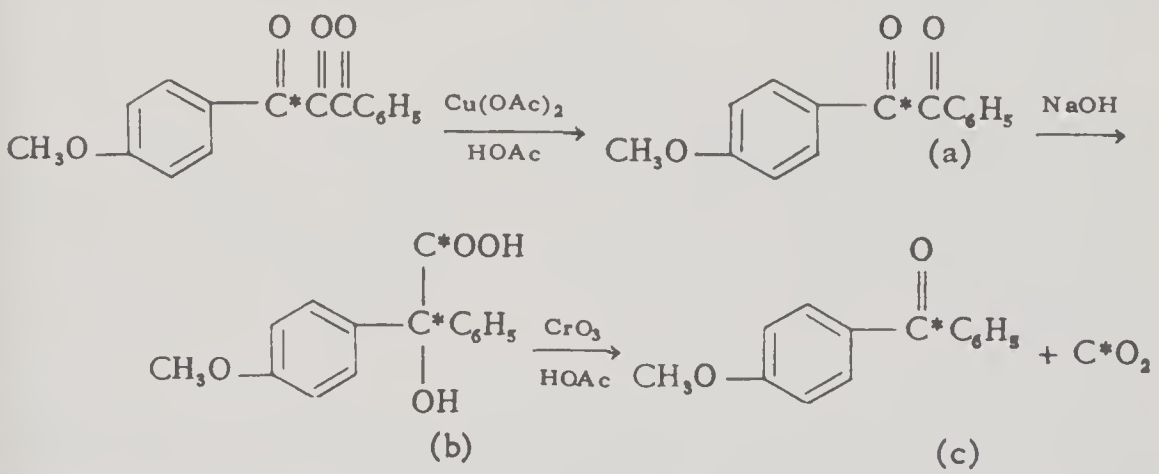
[2] C. Engler and E. Wöhrle, Ber., *20*, 2202 (1887); *Organic Syntheses*, Vol. 23, Wiley, New York, 1943, p. 48.

[3] W. v. E. Doering, T. I. Taylor and E. F. Schoenewaldt, J. Am. Chem. Soc., *70*, 455 (1948).

[4] E. B. Ayers and C. R. Hauser, J. Am. Chem. Soc., *65*, 1095 (1943).

[5] W. Bradley and R. Robinson, J. Chem. Soc., *1928*, 1310.

4-METHOXYBENZILIC-C^{14}_1-α-C^{14}_1 ACID
[(*p*-Methoxyphenyl)phenylglycolic-C^{14}_2 Acid)]



J. D. Roberts, D. R. Smith and C. C. Lee, J. Am. Chem. Soc., 73, 618 (1951).

A. Procedure

(a) *1-p-(Methoxyphenyl)-2-phenylglyoxal-1-C^{14} (C^{14}_1-4-Methoxybenzil).* A mixture of 4.0 g. of 1-(*p*-methoxyphenyl)-3-phenylpropanetrione-1-C^{14}, 8.0 g. of cupric acetate monohydrate and 190 ml. of glacial acetic acid is refluxed for 2 hours in a gentle stream of nitrogen (Note 1). The mixture is diluted with water and extracted with ether; the extract is washed with sodium bicarbonate solution, dried over magnesium sulfate and evaporated to dryness. The yield of product, recrystallized from alcohol, is 3.1 g. (87%), m.p. 61–62°. The molar specific activity is the same as that of the starting material, and degradation studies indicate that there is no isotopic rearrangement (Note 2).

(b) *4-Methoxybenzilic-$C^{14}_{1/1}$-α-$C^{14}_{1/1}$ Acid* (Note 4). A mixture of 1.08 g. of 1-(*p*-methoxyphenyl)-2-phenylglyoxal-1-C^{14} and a solution of 9 g. of sodium hydroxide, in 15 ml. of alcohol and 30 ml. of water, is stirred for 4 days at room temperature. The mixture is diluted with 30 ml. of water and extracted with ether. The aqueous solution is acidified with concentrated hydrochloric acid to precipitate the product, which is collected, washed and dried; yield 1.00 g. (86.2%). The melting point is 148–149°, after recrystallization from benzene, and the molar specific activity is the same as that of the starting material. Degradation studies (see below) indicate that 68.4% of the isotope is at C-2 (Note 4).

(c) *4-Methoxybenzophenone-C^{14}*, (p-*Methoxyphenyl Phenyl Ketone-C^{14}*). A mixture of 0.80 of (*p*-methoxyphenyl)phenylglycolic-$C^{14}_{1/2}$ acid, 10 ml. of acetic acid, 5 ml. of water and 1.0 g. of chromic anhydride is heated for 20 minutes on a steam-bath (Note 5). The mixture is diluted with water, and the product is isolated by ether extraction, vacuum distillation and crystallization from methanol-water; yield 64–70%, m.p. 61–62°. The molar specific activity is 68.4% of that of the starting material (Note 6).

B. Notes

1. The evolved carbon dioxide in the effluent gases was trapped in alkali, precipitated as barium carbonate (90.5% yield), and found to be nonisotopic. It is interesting to note that there is no reaction between carbon monoxide and cupric acetate in acetic acid, although benzoin is oxidized quantitatively to benzil under such conditions (see Note 3).

2. Directions are given by Roberts for oxidizing the C^{14}_1-4-methoxybenzil, with alkaline peroxide, to *p*-anisic-C^{14} acid of the same molar specific activity; yield 66.3%, m.p. 177–179°. Conversion to *p*-anisamide-C^{14} (82% yield, m.p. 164–165°), by successive treatment with thionyl chloride and ammonia in dry ether, and degradation by the Hofmann reaction, gave nonisotopic *p*-anisidine.

Bibenzoyl-C^{14}_1, (C^{14}_1-benzil), is prepared in 75.5% yield (m.p. 93–95°) from diphenylpropanetrione-1-C^{14}, by this same procedure, and recrystallized from carbon tetrachloride. As the molar specific activity is the same as that of the symmetrical starting material, the decarbonylation must involve the central carbon. This decarbonylation has been reported to be brought about by such agents as: acids,[1] bases,[2] sunlight,[3] copper acetate[4] and aluminum chloride.[1,5] In an isotopic experiment[6] employing anhydrous diphenylpropanetrione-1-C^{14} and freshly sublimed aluminum chloride, the products were exclusively nonisotopic carbon monoxide and labeled benzil. In the presence of moisture, however, the products were nonisotopic carbon dioxide and labeled benzoin (80% yield). In an experiment employing sodium hydroxide, the products were nonisotopic carbon dioxide and labeled benzoin (5%) of unchanged molar specific activity.

3. The preparation is incidental to a study of the benzilic acid rearrangement of unsymmetrical α-diketones and the migratory preference of the substituted phenyl group; also see Clark, Hendlye and Neville.[7]

4. This figure corresponds to a ratio of phenyl to *p*-methoxyphenyl migration of 2.17 in the benzilic acid-type rearrangement. In the case of rearrangement at 70° for 15 minutes in 50% aqueous potassium hydroxide (34.8% yield), this ratio appears to be 1.72; the ratio is 1.90 for rearrangement for 10 minutes at 100° (60.5% yield).

5. The liberated carbon-C^{14} dioxide is swept with nitrogen into alkali and precipitated as barium carbonate-C^{14}. As the yield is considerably greater than theory, due to excessive oxidation, the molar specific activity of this diluted carbon-C^{14} dioxide is of no value in determining the degree of phenyl migration.

6. Neville[8] previously reported that the molar specific activity of the degradation product (64% yield) was unchanged from that of the similar 4-methoxybenzilic-α-C^{14} acid (73% yield) and the 2-(*p*-methoxyphenyl)-1-phenylglyoxal-1-C^{14} from which it was formed by rearrangement. This apparent 100% *p*-methoxyphenyl migration was not confirmed by later experiment;[8] see below.

C. Other Preparations

In a similar manner from 2-(*p*-chlorophenyl)-1-phenylglyoxal-1-C^{14}, 2-(*m*-chlorophenyl)-1-phenylglyoxal-1-C^{14}, 2-*p*-tolyl-1-phenylglyoxal-1-C^{14} and 2-(*p*-methoxyphenyl)-1-phenylglyoxal-1-C^{14}, respectively, are prepared[8] the following series of compounds: (*p*-chlorophenyl)phenylglycolic-$C^{14}_{1/2}$ acid (70% yield, m.p. 130°, 67.2% activity at C-2 (earlier experiments[7] gave 61%), 4-chlorobenzophenone-C^{14} (74% yield, m.p. 76°); (*m*-chlorophenyl)phenylglycolic-$C^{14}_{1/2}$ acid (52.1% yield, m.p. 98°, 81.2% activity at C-2), 3-chlorobenzophenone-C^{14} (85.7% yield, m.p. 84°); phenyl-*p*-tolylglycolic-$C^{14}_{1/2}$ acid (66.2% yield, m.p. 133°, 38.8% activity at C-2), 4-methylbenzophenone-C^{14} (93% yield, m.p. 55°); and (*p*-methoxyphenyl)phenylglycolic-$C^{14}_{1/2}$ acid (42.3% yield, m.p. 148°, 31.3% activity at C-2). The latter compound is oxidized[8] with selenium oxide in acetic acid, forming 4-methoxybenzophenone-C^{14} (79% yield, m.p. 61°).

Similarly, from 2-(*o*-chlorophenyl)-1-phenylglyoxal-1-C^{14} are prepared[9] (*o*-chlorophenyl)phenylglycolic-$C^{14}_{1/2}$ acid (44.4% crude yield, m.p. 137.5–138.5° cor. after recrystallization from chloroform-petroleum ether, 31.2% activity at C-2), and 2-chlorobenzophenone-C^{14} (66.6% yield, m.p. 44–45° cor.).

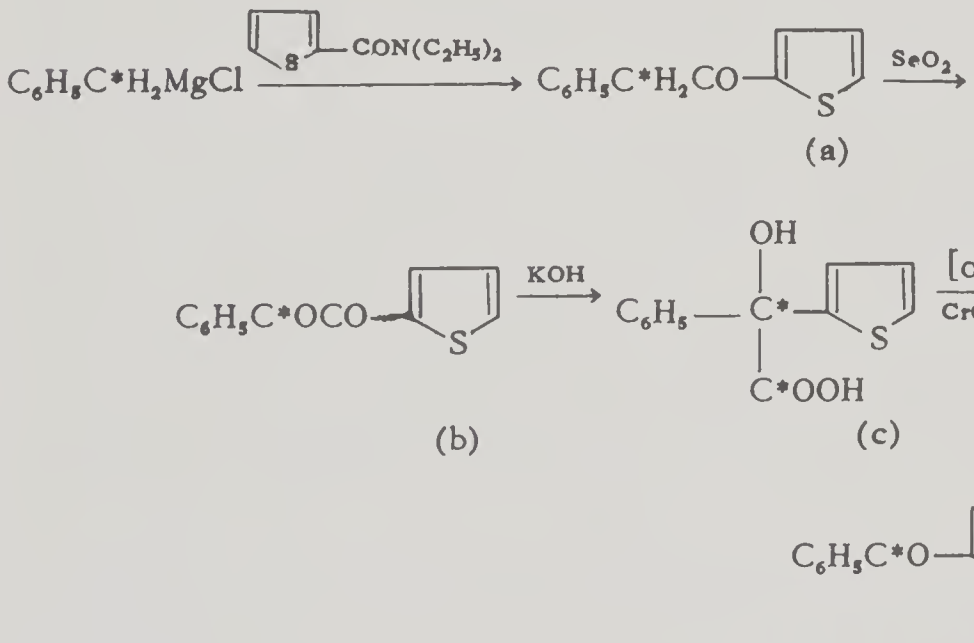
The preparation of 2-(*p*-methoxyphenyl)-1-phenylglyoxal-1-C^{14} has been reported by Neville.[7] Benzaldehyde-C^{14} was condensed with *p*-anisaldehyde in the presence of sodium cyanide (65% yield), and the 4-methoxybenzoin-α-C^{14} was oxidized with copper sulfate and pryidine (100% yield).

The rearrangement of bibenzoyl-C_1^{14} to benzilic-$C_{1/1}^{14}$-α-$C_{1/1}^{14}$ acid, (diphenylglycolic-$C_{1/2}^{14}$ acid) C-2/C-1 = 1.11 ± .01, and the decarboxylation with lead tetraacetate to benzophenone-C^{14} have been reported by Stevens and Attree.[10] This 11% difference in activities is clearly due to isotope effect.

[1]A. Schonberg and R. C. Azzan, J. Chem. Soc., *1939*, 1428.
[2]R. deNeufville and H. v. Pechmann, Ber., *23*, 3375 (1890).
[3]A. Schonberg and A. Mustafa, J. Chem. Soc., *1947*, 997.
[4]A. H. Blatt and W. L. Hawkins, J. Am. Chem. Soc., *58*, 81 (1936).
[5]J. Wegmann and H. Dahn, Helv. Chim. Acta, *29*, 1247 (1946).
[6]J. D. Roberts, D. R. Smith and C. C. Lee, J. Am. Chem. Soc., *73*, 618 (1951).
[7]M. T. Clark, E. C. Handley and O. K. Neville, J. Am. Chem. Soc., 77, 3280 (1955).
[8]O. K. Neville, Atomic Energy Commission Report, BNL-44 (C 10), 49; Nuc. Sci. Abstracts, *4*, 4848 (1950); Nucleonics, 7 (4), 46 (1950); O. K. Neville, *et al.*, Abstracts of Papers, Sixth Southwest Regional Meeting of the Am. Chem. Soc., Dec., 1950, p. 11.
[9]G. G. Smith and D. G. Ott, J. Am. Chem. Soc., 77, 2342 (1955).
[10]W. H. Stevens and R. W. Attree, J. Chem. Phys., *18*, 574 (1950).

PHENYL-2-THIENYLGLYCOLIC-$C_{1/2}^{14}$ ACID

METHOD I



D. G. Ott, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, unpublished work.

A. Procedure (Note 1)

(a) *Benzyl-α-C^{14} 2-Thienyl Ketone* (Note 2). To a stirred Grignard solution prepared from 1.50 g. (61 mmoles) of magnesium, and 7.69 g. (60

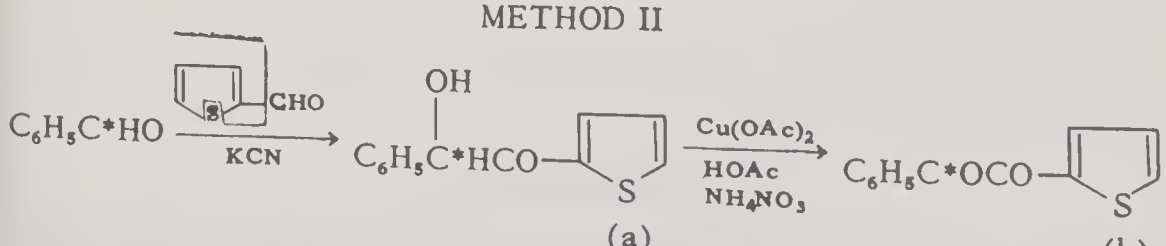
mmoles) of benzyl-α-C^{14} chloride in 60 ml. of ether, is added dropwise a solution of 11.00 g. (60 mmoles) of *N,N*-diethyl-2-thiophenecarboxamide in 150 ml. of absolute ether during 1 hour. The mixture is stored for 2 days and hydrolyzed with 100 ml. of 2.5 *N* hydrochloric acid, the aqueous layer being heated on a steam-bath for 1 hour to complete the reaction. The product is isolated by ether extraction and distilled; yield 8.85 g., b.p. 100–120° (0.35 mm.) (Note 3).

(b) *1-Phenyl-2-(2-thienyl)glyoxal-1-*C^{14} (Note 4). A mixture of 2.80 g. of the crude ketone, 2.0 g. of selenious acid and 6 ml. of wet dioxane is heated on a steam-bath for 20 hours. The precipitated selenium is collected on a filter and washed with dioxane (yield 0.89 g., 81%). The combined filtrate is evaporated under vacuum, and the residue is recrystallized from aqueous methanol; yield 2.02 g. (64.3%), m.p. 64–64.5° from hexane or dilute methanol.

(c) *Phenyl-2-thienylglycolic-*$C^{14}_{1/2}$ *Acid.* (Note 5). A mixture of 0.32 g. (1.55 mmoles) of the diketone, 2 ml. of alcohol and 4.5 ml. of a solution containing 1.6 g. of sodium hydroxide is stirred under nitrogen for 2 days at room temperature in a stoppered flask. The mixture is diluted with water, washed with two 10-ml. portions of ether and acidified with 5 *N* hydrochloric acid. The product is extracted into ether solution (five 10-ml. portions), which is dried over magnesium sulfate, decolorized with carbon and evaporated in a stream of dry nitrogen. The residue is dried under vacuum over phosphorus pentoxide; yield 0.36 g. (99%), m.p. 126–127° (dec.). Degradation studies, see below, indicate that 28.4% of the radioactivity is located at C-2.

(d) *Phenyl 2-Thienyl Ketone-*C^{14}. A mixture of the hydroxyacid in 10 ml. of acetic acid, and 0.60 g. of chromium trioxide in 6 ml. of water is heated for 30 minutes on a steam-bath, then diluted with 35 ml. of water and extracted with four 10-ml. portions of ether. The extract is washed with water, 3 *N* sodium hydroxide and water, then decolorized with carbon, dried over magnesium sulfate and evaporated. The colorless oil is dried under vacuum (93% yield based on the diketone) and crystallized by trituration with hexane at −20°. The product melts at 55–56° after repeated recrystallization from hexane; the molar specific activity is 28.4% of that of the original starting material.

METHOD II



D. G. Ott, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, unpublished work.

(a) *α-Hydroxybenzyl-α-C^{14} 2-Thienyl Ketone* (Note 6). To a solution of 5.00 g. (47.1 mmoles) of benzaldehyde-C^{14} and 5.77 g. (47.1 mmoles) of 2-thiophenecarboxaldehyde (Note 7) in 25 ml. of alcohol is added a solution of 3.6 g. of potassium cyanide in 10 ml. of water. The mixture is refluxed for 1.5 hours, then diluted with 60 ml. of 50% alcohol (Note 8) and cooled to 10°. The pale-orange product is collected on a filter and washed successively with 50% alcohol, water, saturated sodium bicarbonate solution, dilute potassium carbonate solution and water; yield 5.68 g. (54%), m.p. 132–133° (Note 9).

(b) *1-Phenyl-2-(2-thienyl)glyoxal-1-C^{14}*. A stirred mixture of 2.43 g. (11 mmoles) of the hydroxy ketone, 0.025 g. of cupric acetate, 1.2 g. of ammonium nitrate and 8.5 ml. of 80% aqueous acetic acid is refluxed for 2 hours, cooled, seeded, diluted with water to completely precipitate the product, and stored in a refrigerator. The pale-yellow product is collected and washed with water; yield 2.24 g. (96%), m.p. 59–60° (Note 10).

B. Notes

1. The preparations are incidental to a tracer study of migratory preferences in benzilic acid rearrangements in the heterocyclic series; also see 4-methoxybenzilic-$C^{14}_{1/1}$-α-$C^{14}_{1/1}$ acid and 2-chloro-9-fluorenone-9-C^{14}.

2. The procedure is an adaptation of the method of Suter[1] for preparing deoxybenzoins; also see 2-(*p*-chlorophenyl)acetophenone-1-C^{14}.

3. Infrared analysis of the distillate indicates that the product separates as a colorless solid from what appears to be a mixture of the ketone and the amide, which does not interfere in the oxidation step.

4. See Riley and Gray[2] and 1-(*p*-chlorophenyl)-2-phenylglyoxal-2-C^{14}.

5. The prodedure is a modification of that of Biel.[3]

6. The procedure is an adaptation of the benzoin condensation[4] and benzilic rearrangement described by Biel.[3]

7. Directions for preparing this intermediate are given by Weston and Michaels.[5]

8. The crude product may be precipitated with water in almost quantitative yield, but the brown coloration is more readily removed in the described manner.

9. Cream-colored plates, m.p. 133–134°, are formed after two recrystallizations from 50% alcohol.

10. The melting point is raised to 62–63° by three recrystallizations from methanol and three recrystallizations from hexane.

C. Other Preparations

From *N,N*-diethyl-3-thiophenecarboxamide, by Method I, are prepared:[6] benzyl-α-C^{14} 3-thienyl ketone (yield and boiling range same as for the

2-isomer), 1-phenyl-2-(3-thienyl)glyoxal-1-C^{14} (69% yield, m.p. 43–43.5° from dilute methanol), phenyl-3-thienylglycolic-$C^{14}_{1/2}$ acid (99% yield, m.p. 131–132°) and phenyl 3-thienyl ketone-C^{14} (96% yield based on the diketone, m.p. 63–64°, molar specific activity 16.9% of that of the starting material).

From 3-thiophenecarboxaldehyde,[7] by Method II, are prepared: α-hydroxybenzyl-α-C^{14} 2-thienyl ketone (63% yield, m.p. 113–114°) and 1-phenyl-2-(3-thienyl)glyoxal-1-C^{14} (97% yield, m.p. 40–45°; repeated recrystallization from methanol failed to yield much improvement of the melting point, but recrystallization from hexane raised the melting point to 79–80°).

[1]C. M. Suter and A. W. Weston, J. Am. Chem. Soc., *61*, 232 (1939); S. S. Jenkins, J. Am. Chem. Soc., *55*, 703, 1618, 2896 (1933).

[2]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 510, Note 3.

[3]J. H. Biel, E. P. Sprengeler, H. A. Leiser, J. Horner, A. Drukker and H. L. Friedman, J. Am. Chem. Soc., 77, 2250 (1955); E. Campaigne and R. C. Bourgeois, J. Am. Chem. Soc., 75, 2702 (1953).

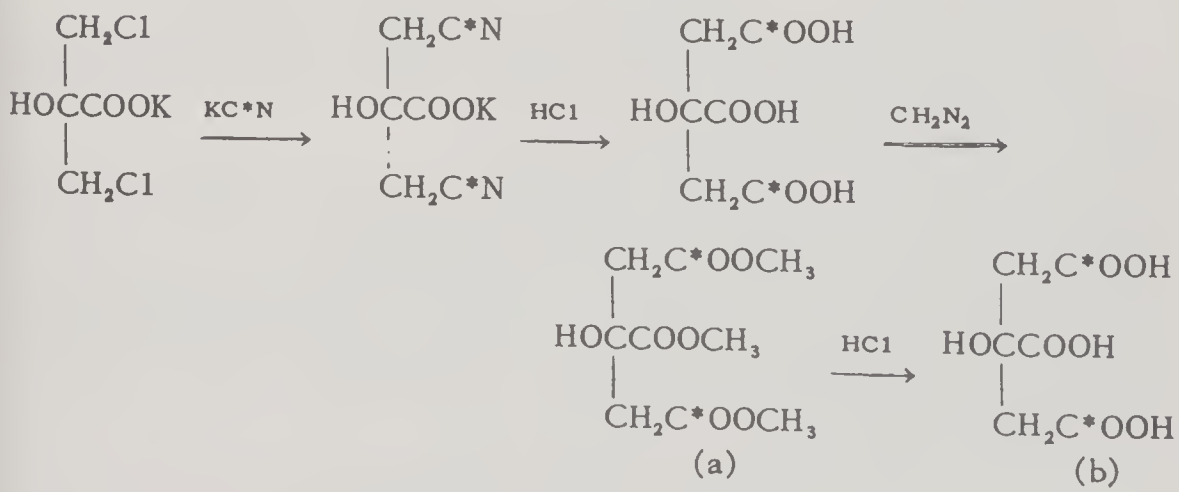
[4]*Organic Reactions*, Vol. IV, Wiley, New York, 1948, p. 269.

[5]*Organic Syntheses*, Vol. 31, Wiley, New York, 1951, p. 108.

[6]D. G. Ott, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, unpublished work.

[7]*Organic Syntheses*, Vol. 33, Wiley, New York, 1953, p. 93.

CITRIC-1, 5-C^{14}_2 ACID



S. Rothchild and M. Fields, J. Am. Chem. Soc., 74, 2401 (1952).

A. Procedure (Note 1)

(a) *Methyl Citrate-1, 5-C^{14}_2.* To a solution of 3.91 g. of potassium cyanide-C^{14} in 10 ml. of water maintained at 75° is added, over a period

of 10 minutes, a solution of 5.17 g. of bis(chloromethyl)glycolic acid (Note 2) and 2.07 g. of potassium carbonate in 7 ml. of water. The mixture is heated for 15 minutes, then cooled, acidified with hydrochloric acid and evaporated to dryness at reduced pressure.

The residual potassium bis(cyano-C^{14}-methyl)glycolate is hydrolyzed by refluxing overnight with 50 ml. of concentrated hydrochloric acid. The mixture is evaporated to dryness at reduced pressure, the residual mineral acid being removed with the evaporation of several portions of added water. To the dry, ice-cooled residue is added, with stirring, 450 ml. of an ethereal solution of diazomethane[1] prepared from 30 g. of nitrosomethylurea (Note 3). After several hours, the solution is filtered and dried, and the solvent is distilled. The dark oil yields a nearly colorless crystalline product, when taken up in several ml. of isopropyl ether; yield 3.11 g. (44%), m.p. 76–77°.

(b) *Citric-1, 5-C_2^{14} Acid.* A mixture of 1.99 g. of methyl citrate-1,5-C_2^{14} and 20 ml. of concentrated hydrochloric acid is refluxed for several hours and evaporated to dryness; then the residue is dissolved in water, decolorized with charcoal and evaporated to dryness. A solution of the residue in 5 ml. of acetone is diluted with 10 ml. of benzene and warmed to distill the acetone, until crystallization commences; yield 1.48 g., m.p. 148–150°. An additional 0.080 g. obtained from the mother liquor raises the total yield to 96% (Note 4). The molar specific activity agrees with twice that of the starting cyanide.

B. Notes

1. The procedure is a modification of the method of Grimaux.[2]

2. Directions are given by Rothchild and Fields for preparing the starting material by hydrolyzing the condensation product from hydrogen cyanide and 1,3-dichloro-2-propanone.

3. The citric acid is isolated through its methyl ester, and easily purified crystalline solid with a sharp melting point. This method is superior to the usual method employing the calcium salt.[3]

4. Neutralization equivalent 63.8, calc. 64.0. The monohydrate loses water of crystallization at 40–50°. A degradation of citric acid is described by Lewis[3] and by Mosbach.[4]

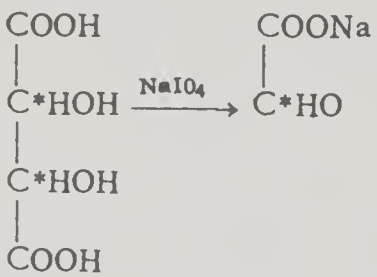
C. Other Preparations

By a similar procedure, starting with L-2-chloromethyl-2-hydroxysuccinic acid, is prepared[5] (asymmetrically labeled) sodium citrate-1-C^{14}. The yield of the intermediate calcium citrate-1-C^{14} is 23% from the carboxylic acid and 32% from the methyl ester by the same procedure.

The product was characterized by (1) plotting the curves of radioactivity and acid titer of a silica-gel chromatogram,[6] (2) colorimetric assay by a method which is specific[7] for citric acid (and acetonedicarboxylic acid). The asymmetric distribution of the isotope about the tertiary carbon of citric acid is indicated by biochemical degradation.

[1]*Organic Reactions,* Vol. I, Wiley, New York, 1942, p. 50.
[2]E. Grimaux and P. Adam, Bull. soc. chim. France, (2) *36,* 18 (1881).
[3]K. F. Lewis and S. Weinhouse, J. Am. Chem. Soc., *73,* 2500 (1951).
[4]E. H. Mosbach, E. F. Phares and S. F. Corson, Arch. Biochem. Biophys., *33,* 179 (1951).
[5]P. E. Wilcox, C. Heidelberger and V. R. Potter, J. Am. Chem. Soc., *72,* 5019 (1950); Nucleonics, *7* (3), 57 (1950).
[6]F. A. Isherwood, Biochem. J., *40,* 688 (1946); L. M. Marshall, J. M. Orten and A. H. Smith, J. Biol. Chem., *179,* 1127 (1949).
[7]S. Natelson, J. K. Lugovoy and J. B. Pincus, J. Biol. Chem., *170,* 597 (1947).

SODIUM GLYOXYLATE-2-C^{14}



A. Weissbach and D. B. Sprinson, J. Biol. Chem., *202,* 1023 (1953).

Procedure

To a solution of 0.5 g. (3.3 mmoles) of tartaric-2,3-C_2^{14} acid in 10 ml. of water is added 7.8 ml. of 0.42 *N* sodium periodate and, after 10 minutes the solution is extracted continuously with ether for 24 hours. The ether solution is extracted with a solution of 0.340 g. (4 mmoles) of sodium bicarbonate in 20 ml. of water and washed with 10 ml. of water. The combined aqueous solution is adjusted to pH 7.0 with sodium bicarbonate, then concentrated under reduced pressure to 1–2 ml., and treated with absolute alcohol to precipitate the product, and the mixture is stored overnight in a refrigerator. The product is collected by centrifuging and washed with alcohol. The yield is 0.442 g.; an additional 0.158 g. is obtained from the mother liquors, making the total yield 80%. The 2,4-dinitrophenylhydrazone melts at 191°.

(ETHYLTHIO)ACETIC-1-C^{14} ACID

$$BrCH_2C^*OONa \xrightarrow[(H_2SO_4)]{C_2H_5SNa} C_2H_5SCH_2C^*OOH$$

A. E. Almond, A. M. Burditt, D. E. Douglas and J. Eccles, Can. J. Chem., 33, 679 (1955).

A. Procedure (Note 1)

The crude bromoacetic-1-C^{14} acid prepared from 4.8 mmoles of sodium acetate-1-C^{14} is adjusted to pH 8 with dilute sodium hydroxide and evaporated to dryness. The residue is dissolved in a minimum volume of water and treated with 10 ml. of 0.62 *M* sodium ethanethiolate in alcohol. The mixture is stored for 24 hours, refluxed for 3 hours and evaporated to dryness under vacuum. The residue is dissolved in water, and the solution is continuously extracted with ether for 45 hours. The aqueous solution is adjusted to pH 2 with sulfuric acid and extracted overnight with ether. The extract is dried over sodium sulfate and concentrated in a stream of nitrogen, and the residue is distilled under high vacuum with a bath at 100°; yield 0.1555 g. (21%), n_D^{22} 1.4800 (Note 2). Purity is established by paper chromatography and radioautography (Note 3).

B. Notes

1. The procedure is a modification of that described by Ramberg.[1]

2. (Ethylthio)acetic acid boils[1] at 117–118° (11 mm.), d_{20}^{20} 1.1518.

3. An ascending paper chromatogram is developed on Whatman No. 1 paper with 1-butanol-water-ammonia, R_f 0.34. The dried paper is sprayed with 0.1% aqueous chloramine-T, dried and sprayed immediately with starch-potassium iodide solution. The presence of mercapto acids is indicated by white spots on a blue background.

[1]L. Ramberg, Ber., *40*, 2588 (1907).

3-(ETHYLTHIO)PROPIONIC-1-C^{14} ACID

$$C_2H_5SCH_2CH_2Cl \xrightarrow{NaC^*N} C_2H_5SCH_2CH_2C^*N \xrightarrow{HCl} C_2H_5SCH_2CH_2C^*OOH$$

A. E. Almond, A. M. Burditt, D. E. Douglas and J. Eccles, Can. J. Chem., 33, 679 (1955).

A. Procedure

A mixture of 5.4 mmoles of sodium cyanide-C^{14}, 8 ml. of 80% ethanol and 5.4 mmoles (0.65 ml.) of ethyl 2-chloroethyl sulfide is refluxed for 4

hours, then evaporated under vacuum. The residual ethyl 3-(ethylthio)-propionitrile-1-C^{14} is heated with 5 ml. of 6 *N* hydrochloric acid and 2.5 ml. of glacial acetic acid at 100° for 12 hours in a sealed tube. The mixture is evaporated under vacuum, and the residue is dissolved in water and extracted with chloroform. The chloroform solution is extracted with dilute sodium hydroxide and water; then the alkaline extract is adjusted to pH 2 and extracted with four portions of chloroform. The extract is dried over sodium sulfate and concentrated, and the product is distilled under high vacuum; yield 0.2612 g. (36%), $n_D^{21.5}$ 1.4805.

B. Other Preparations

In the same manner from sodium cyanide-C^{14} and ethyl 3-chloropropyl sulfide is prepared 4-(ethylthio)butyric-1-C^{14} acid; yield 12% n_D^{22} 1.4805. The yield from carbonation of 3-(ethylthio)propylmagnesium bromide with carbon-C^{14} dioxide is 53%.

C*-GLYCINE

METHOD I

$$ClCH_2C^*OOH \xrightarrow[NH_4OH]{(NH_4)_2CO_3} NH_2CH_2C^*OOH$$

B. M. Tolbert and D. M. Hughes, Atomic Energy Commission Report, URCL-705; Nuc. Sci. Abstracts, 4, 5207 (1950).

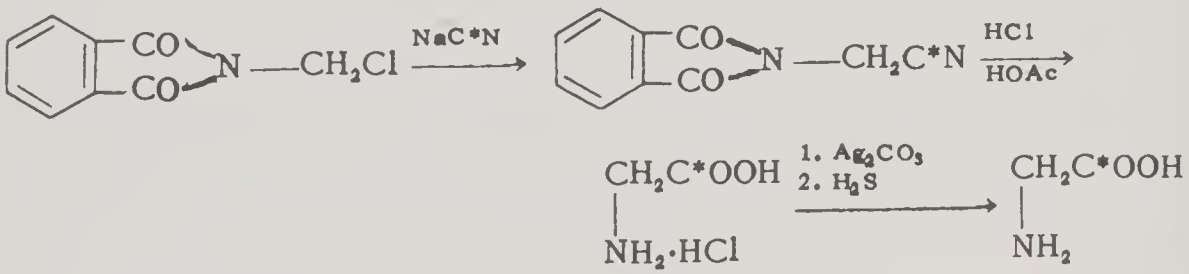
A. Procedure (Note 1)

Glycine-1-C^{14}. To a mixture of 15 ml. of ammonium hydroxide and 7.5 g. of freshly ground ammonium carbonate, maintained at 60–65° in a 60-ml. two-necked flask fitted with a reflux condenser and dropping funnel, is added, over 30 minutes in 3 ml. of aqueous solution, the chloroacetic-1-C^{14} acid prepared from 0.6–1.0 g. of dry sodium acetate-1-C^{14}. The mixture is heated for 8–12 hours, then distilled to dryness at the pressure of a water pump and with a final bath temperature of 115° (Note 2). The crude salt is dissolved in water and placed upon an ion exchange column of Dowex-50 (Note 3), and the column is washed with 500 ml. of water (Note 4). The product is eluted from the column with 250 ml. of 1.5 *N* ammonium hydroxide (Note 5), followed by 250 ml. of water. The combined eluate is evaporated to dryness, and the residue is dissolved in 25–40 ml. of water. The solution is adjusted to pH 11.5 with dilute sodium hydroxide, then covered with a watch glass and boiled down to half volume. The volume is restored with water, the pH is adjusted, and

the boiling is repeated. The solution is adjusted to pH 5 with dilute hydrochloric acid (Note 6) and evaporated to dryness. The residue is dried at 80° (0.1μ), then sublimed at 0.1–0.01μ by heating at 150° for 3 hours and 200° for 2 hours. The path is 1 cm., and the condenser is cooled with liquid nitrogen (Note 7). The residue is dissolved in water, the pH is readjusted, the solution is evaporated, and the sublimation is continued. Resublimation of the combined sublimates gives the pure product in 50–60% yield (occasionally 60–70%) based on sodium acetate (Note 8). Analysis by two-dimensional paper chromatography and radioautography shows the product to be homogeneous (Note 9).

Glycine-2-C^{14} is prepared by the same procedure, starting with chloroacetic-2-C^{14} acid.

METHOD II



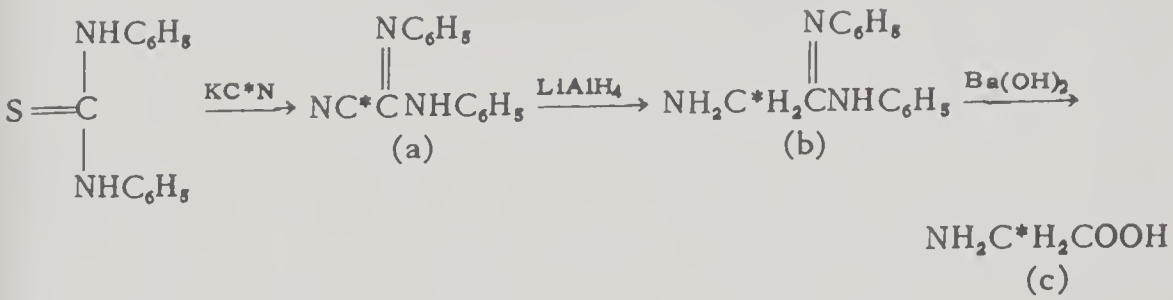
W. Sakami, W. E. Evans and S. Gurin, J. Am. Chem. Soc., 69, 1110(1947).

A. Procedure

Glycine-1-C^{13}. To a solution of 0.50 g. of sodium cyanide-C^{13} in 25 ml. of acetone-free methanol is added a solution of 2.0 g. of *N*-(chloromethyl)phthalimide in 1 ml. of warm dioxane (Note 10). The mixture is set aside for 2 hours, then evaporated to dryness, and the residue is extracted with successive 15-, 10- and 5-ml. portions of warm dioxane. The combined solutions are evaporated, and the residue is refluxed for 15 hours with a mixture of 11 ml. of acetic acid, 13 ml. of water and 12 ml. of concentrated hydrochloric acid. The mixture is cooled to 0°, the phthalic acid is filtered off, and the filtrate is evaporated to dryness under vacuum. The residue is treated in aqueous solution with excess silver carbonate to remove chloride (Note 11). The combined filtrate and washings are concentrated to about 25 ml., then saturated with hydrogen sulfide and filtered. The combined filtrate and washes are evaporated to dryness; the residue is taken up in a few ml. of water and diluted with ethanol to precipitate the product, which is filtered off, washed with alcohol and dried; yield 0.62 g. (81%), m.p. 238–239°(Note 12).

METHOD III

GLYCINE-2-C^{13}



G. Ehrensvärd and R. Stjernholm, Acta Chem. Scand., 3, 971 (1949).

A. Procedure

(a) *1-Cyano-C^{13}-N,N′-diphenylformamidine.* To a solution of 7 g. of potassium cyanide-C^{13} in 20 ml. of water is added 30 g. of basic lead carbonate, 20 g. of 1,3-diphenyl-2-thiourea, (thiocarbanilide), and 50 ml. of alcohol. The mixture is stirred and heated to 50–55° for 1 hour (Note 13). When the reaction is complete, 200 ml. of cold water is added, and the precipitate is filtered off and washed with cold water. The residue is leached with 200 ml. of boiling 95% alcohol, and the filtered solution is evaporated to dryness (Note 14); yield 19 g. (90%).

(b) *2-Amino-N-N′-diphenylacetamidine-2-C^{13}.* To a solution of 16 g. of lithium aluminum hydride[1] in 500 ml. of absolute ether contained in a 2-l. three-necked flask equipped with a reflux condenser, dropping funnel, mercury-sealed stirrer, and nitrogen inlet, is added a solution of 19 g. of the crude formamidine in 300 ml. of absolute ether. This addition is made over a period of about 2 hours in a slow stream of dry nitrogen and at such a rate as to maintain a gentle reflux (Note 15). The mixture is stirred for another 20 minutes, then cooled in an ice-bath, and hydrolyzed by the dropwise addition of 500 ml. of water. The aqueous layer is separated and extracted with three 100 ml. portions of ether. The combined extract is dried over sodium sulfate and evaporated to dryness, leaving 17.5 g. of yellow, oily, crystalline material (Note 16).

(c) *Glycine-2-C^{13}.* The crude acetamidine (16.5 g.) is heated under reflux for 1 hour at 130° with 80 ml. of 10% barium hydroxide solution (Note 17). The mixture is cooled and extracted with three 50-ml. portions of ether. The aqueous solution is freed of barium ions by addition of the stoichiometric amount of 10% sulfuric acid, the barium sulfate being removed by centrifuging and washing with boiling water. The combined solution is concentrated to 10 ml. and decolorized with charcoal, and

the product is precipitated by dilution with five volumes of ethanol and cooling to 0°. The colorless crystals are collected and washed with absolute alcohol; yield 3.8 g. (Note 18). Degradation studies indicate that the isotope is confined to C-2 (Note 19).

B. Notes

1. The procedure is a modification of the isotopic procedure described by Ostwald;[2] also see Cheronis,[3] and Orten and Hill.[4] The bromoacetic acid has been employed alternatively;[5,6,7,8] yield 55–60%.[6]

2. The evolution of carbon dioxide prevents bumping. The last traces of moisture are removed under high vacuum.

3. A column (20 × 15 cm.) containing 60 cc. of 20–40-mesh resin is prepared by recycling the resin alternately with 1.5 *N* sodium hydroxide and 1.5 *N* hydrochloric acid, washing thoroughly with water after each treatment, and ending with acid. Although the column has a working capacity of 0.8 g. of crude glycine salt, it is preferable not to exceed 0.4 g. per column.

4. The effluent, collected during 4 hours, contains about 25% of the initial activity but no glycine.

5. The use of excess ammonium hydroxide saturates the column with NH_4^+, shifting the equilibrium of the other cations so that they are washed out. This procedure gives an eluate containing only the product and volatile ammonia, obviating the appearance of ammonium chloride which would have been formed by elution with hydrochloric acid.

6. If the solution is adjusted to the isoelectric point (pH 6.1), the sublimation residue tests basic.

7. As the sublimate is light, flaky and apt to be electrostatically charged, it is best removed wet.

8. Ostwald[2] has obtained the product (m.p. 225° dec.) in 79% yield based on chloroacetic-1-C^{14} acid, by isolating and purifying the product through crystallization from methanol.

9. The chromatogram is developed by the method of Benson[9] and, following preparation of the radioautogram, is sprayed with ninhydrin solution to locate the α-amino acids.

10. Directions are given by Sakami for preparing the starting material by treating *N*-(hydroxymethyl)phthalimide[10] with excess thionyl chloride.

11. A modification of this method has been used by Amstein,[11] who isolated the glycine-1-C^{14} from an aqueous-ethanol solution of the hydrochloride by addition of pyridine.

12. Titration with perchloric acid shows the molecular weight to be 75.2 (theory 75.1). Bond[12] has reported a yield of 62.5% from 2.68 g. of potassium cyanide-C^{13} by this procedure, m.p. 236–237° (dec.); Heyns,[13]

66.5%. The method has been used by Shemin[14] for the preparation of glycine-1-C^{13}-N^{15}.

13. The reaction is complete when the filtrate from a small aliquot does not discolor a small amount of lead carbonate (PbS) at short boiling.

14. The pure product may be obtained in at least 80% yield by crystallization from about 50 ml. of alcohol at 0°; light yellow plates, m.p. 139°. The crude material is satisfactory for the next step, provided it is alcohol-free.

15. A yellow precipitate, formed at the beginning of the reaction, graaually turns white.

16. The product is contaminated with a small amount of aniline. Purification is accomplished by treating 1 g. of the crude material with 2 ml. of methanol and filtering, m.p. 106°; the melting point rises to 112° after recrystallization from petroleum ether. The yield is 65–70%.

17. Aniline is seen to separate from the water phase after 15 minutes. Hydrolysis with concentrated hydrochloric acid at 100° is very slow.

18. The yield is 48% based on cyanide, but higher yields have been obtained. The melting point of ethyl glycine ester hydrochloride is 143°.

19. Degradation is effected by decarboxylation with ninhydrin.[15] Analysis of the liberated carbon dioxide is compared with that obtained from total combustion of a sample of the glycine.

C. Other Preparations

The preparation of glycine-1-C^{13}-2-C^{14}, glycine-1-C^{14} and glycine-2-C^{14} by a modification[6,8] of Method I, employing the bromoacetic acid, has been reported by Weissbach,[5] Bloch[6] and Elwyn,[7] respectively; yield 55–60% based on potassium acetate.[6] The preparation of glycine-2-C^{14} has been described by Kögl.[8]

N-Acetylglycine-1-C^{14} has been prepared by Bloch;[6] see Bloch and Rittenberg.[16]

[1]R. Nystrom and W. G. Brown, J. Am. Chem. Soc., *70*, 3738 (1948).
[2]R. Ostwald, J. Biol. Chem., *173*, 207 (1948).
[3]N. D. Cheronis and K. H. Spitzmueller, J. Org. Chem., 6, 349 (1941).
[4]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 300.
[5]A. Weissbach and D. B. Sprinson, J. Biol. Chem., *203*, 1031 (1953).
[6]K. Bloch, J. Biol. Chem., *179*, 1245 (1949).
[7]D. Elwyn, A. Weissbach, S. S. Henry and D. B. Sprinson, J. Biol. Chem., *213*, 281 (1955).
[8]F. Kögl and A. M. Municio, Hoppe-Seyler's Z. physiol. Chem., *300*, 6 (1955).
[9]A. A. Benson, J. A. Bassham, M. Calvin, V. A. Haas, T. C. Goodale and W. Stepka, J. Am. Chem. Soc., *72*, 1710 (1950).
[10]F. Sachs, Ber., *31*, 3230 (1898).
[11]H. R. V. Arnstein, Biochem. J., *48*, 27 (1951).
[12]H. W. Bond, J. Biol. Chem., *175*, 531 (1948).
[13]K. Heyns and R. Brockmann, Z. Naturforsch., *9b*, 21 (1954).
[14]D. Shemin, J. Biol. Chem., *162*, 297 (1946).

[15]D. D. Van Slyke, R. T. Dillon, D. A. MacFadyen and P. Hamilton, J. Biol. Chem., *41*, 627 (1941).

[16]K. Bloch and D. Rittenberg, J. Biol. Chem., *169*, 467 (1947).

ALANINE-1-C^{14}

$$CH_3CH_2C^*OONa \xrightarrow{HCl} CH_3CH_2C^*OOH \xrightarrow[P]{Br_2} CH_3\underset{\displaystyle Br}{\underset{|}{C}}HC^*OOH \xrightarrow[NH_4OH]{(NH_4)_2CO_3}$$

(a)

$$CH_3\underset{\displaystyle NH_2}{\underset{|}{C}}HC^*OOH$$

(b)

R. Ostwald, P. T. Adams and B. M. Tolbert, J. Am. Chem. Soc., *74*, 2425 (1952).

A. Procedure

(a) *2-Bromopropionic-1-C^{14} Acid* (Note 1). An evacuated bulb (10 μ) containing 0.937 g. of dry sodium propionate-1-C^{14} is filled with an excess of dry, purified hydrogen chloride (to a pressure of about one-half atmosphere) and closed off. The solid is heated gently over a burner until the exchange reaction is complete; then the mixture is vacuum-distilled into a large trap cooled with liquid nitrogen (Note 2). The cooling bath is exchanged for a Dry Ice-isopropyl alcohol mixture, and the excess hydrogen chloride is pumped off. The propionic-1-C^{14} acid is distilled into a bromination flask (Note 3) containing 0.04 g. of red phosphorus, 0.02 g. of iodine and 0.2 ml. of propionyl chloride. The mixture is refluxed on a steam-bath for 30 minutes to destroy any water; then 1.5 ml. of bromine is added dropwise, and heating is continued for 3 hours. The excess bromine is removed in a gentle stream of air; then the cooled mixture of acid and acid halide is hydrolyzed by the slow addition of 2 ml. of water.

(b) *Alanine-1-C^{14}* (Note 4). The crude 2-bromopropionic-1-C^{14} acid is added slowly to a mixture of 6 g. of ammonium carbonate and 15 ml. of concentrated ammonium hydroxide. The mixture is maintained at 60° for 6 hours, then evaporated to dryness under vacuum (Note 5). A solution of 0.6–0.9 g. of the residue in a minimum amount of water is put on a cation-exchange column (Note 6), and the anions are washed out with 500 ml. of water. The product is eluted with 250 ml. of 1.5 *N* ammonium hydroxide, followed by 125 ml. of water. The residue, obtained by

evaporating the eluate to dryness, contains 88% of the activity put on the column (Note 7). The residue is made slightly alkaline and evaporated to dryness to remove traces of ammonia. The residue is redissolved in water, and the solution is adjusted to pH 6.8 and evaporated to dryness in a vacuum sublimator. The product is sublimed at 160–200° (2 hours) at 1 μ. and collected on a cold finger cooled by liquid nitrogen. The residue is redissolved, the pH is again adjusted to 6.8, the solution is evaporated, and the sublimation is continued (Note 8). The yield is 0.802 g. (76%) and the radiochemical yield is 80.5% (Note 9). Purity and identity are checked by two-dimensional paper chromatography (Note 10) and radioautography.

B. Notes

1. The procedure is a modification of the method of Eck and Marvel.[1]

2. The residual salt contains less than 0.1% of the initial activity and no demonstrable amount of liberated acid. The propionic acid contains 3–5% water.

3. The flask is fitted with an addition tube and a low-temperature condenser cooled with Dry Ice-isopropyl alcohol (see leucine-2-C^{14}).

4. The amination procedure is adapted from the general method of Cheronis and Spitzmueller;[2] also see Tobie and Ayers.[3]

The product has been used to establish the fact that the conversion of α-amino acids to α-acylamino ketones, by means of the Dakin-West reaction, takes place with the liberation of carbon dioxide furnished solely by the amino acid.[4]

5. The distillate contains 1–2% of the initial activity.

6. A glass column (30 cm. × 2 cm. o.d.) filled with 60 ml. of 20–40 mesh Dowex-50 is treated by cycling it to exhaustion three or four times successively with 2 *N* sodium hydroxide and 2 *N* hydrochloric acid, ending with the acid, then washing thoroughly with water.

7. The two residues, obtained by separately evaporating the aqueous effluate and a hydrochloric acid-regenerating eluting solution, contain 3% and 5% of the initial activity, respectively.

8. The residue contains 4% of the initial activity.

9. The product may be decarboxylated by bacterial action, if left standing at room temperature in unsterilized solution.

10. The two solvent systems[5] used are phenol saturated with water and 1-butanol-propionic acid-water (2:1:1.4), respectively. The radioautograph spot corresponds with the ninhydrin spot produced by spraying the paper with a 0.1% alcoholic solution of the reagent. The systems phenol-water-ammonia[6] and pyridine-water[7] have been used by Busch.[8]

The effect of pH on the adherence of alanine-1-C^{14} to Dowex-1 is described quantitatively by Busch.[8,9] The derivative *N*-(2,4-dinitro-

phenyl)alanine-1-C^{14} was prepared[8,10] and recrystallized by the method of Porter[11] for assay purposes; alanine-1-C^{14} 3,5-dinitrobenzoyl ester, also, was prepared.[8,12]

C. Other Preparations

Alanine-2-C^{14} is prepared, by a similar procedure, in 63% radiochemical yield and 67% chemical yield (0.689 g.) based on the sodium propionate-2-C^{14} (0.935 g.) and propionyl chloride used.

Alanine-3-C^{14}, likewise, is prepared in 74% radiochemical yield and 77% chemical yield (0.965 g.) based on the sodium propionate-3-C^{14} (1.11 g.) and propionyl chloride used.

Alanine-1-C^{14} has been prepared by Loftfield,[13] by a Strecker synthesis[14] from hydrogen cyanide-C^{14}, acetaldehyde and ammonia. The yield at the 1-mmole level was 0.27 g. (after adding 0.30 g. of carrier alanine), and the radiochemical yield was about 38%.

Alanine-1-C^{13} has been prepared in about 48% yield by Gurin,[15] also employing the Strecker synthesis.

Alanine-3-C^{14} has been prepared by Ostwald[16] through condensation of methyl-C^{14} iodide with ethyl acetamidomalonate. However, the product was contaminated with about 10% of a compound believed to be *N*-methyl-C^{14}-glycine.

The preparation of D-alanine-1-C^{14} has been reported by Snell.[17] *N*-acetyl-DL-alanine-1-C^{14} (m.p. 136°) was prepared and converted to *N*-acetyl-D-alanine-1-C^{14} by a combination of the asymmetric enzymatic hydrolytic methods (D-amino acid oxidase) of Fodor[18] and Baker.[19] Hydrolysis with hydrobromic acid gave the final product, containing less than 1.1% of L-alanine-1-C^{14}; a determination of D-alanine is described.

[1]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 74.
[2]N. D. Cheronis and K. H. Spitzmueller, J. Org. Chem., 6, 349 (1941).
[3]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 23.
[4]A. Fry and H. Rappoport, Atomic Energy Commission Report, UCRL-877; Nuc. Sci. Abstracts, *4*, 6594 (1950); C. S. Rondestvedt, Jr., B. Manning and S. Tabibian, J. Am. Chem. Soc., *72*, 3183 (1950).
[5]A. A. Benson, J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas and W. Stepka, J. Am. Chem. Soc., *72*, 1710 (1950).
[6]R. Consden, A. H. Gordon and A. J. P. Martin, Biochem. J., *38*, 224 (1944).
[7]H. K. Berry, H. E. Sutton, L. Cain and T. Berry, The Univ. of Texas Publication 5109, pp. 22–55 (1951).
[8]H. Busch, Cancer Research, *15*, 365 (1955).
[9]H. Busch, Cancer Research, *13*, 789 (1953).
[10]F. Sanger, Biochem. J., *39*, 507 (1945).
[11]R. R. Porter, *Methods in Medical Research*, Vol. 3, Year Book Publishers, Chicago, 1950, p. 259.
[12]H. D. Cheronis and J. B. Entrikin, *Semimicro Qualitative Organic Analysis*, Crowell, New York, 1947, p. 273.

[13]I. D. Frantz, Jr., R. B. Loftfield and W. D. Miller, Science, *106*, 544 (1947); R. B. Loftfield, Nucleonics, *1* (3), 54 (1947).

[14]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 21.

[15]S. Gurin, A. M. Delluva and D. W. Wilson, J. Biol. Chem., *171*, 101 (1947).

[16]R. Ostwald, P. T. Adams and B. M. Tolbert, J. Am. Chem. Soc., *74*, 2425 (1952).

[17]E. E. Snell, N. S. Rodin and M. Ikawa, J. Biol. Chem., *217*, 803 (1955).

[18]P. J. Fodor, V. E. Price and J. P. Greenstein, J. Biol. Chem., *178*, 503 (1949); V. E. Price, J. B. Gilbert and J. P. Greenstein, J. Biol. Chem., *179*, 1169 (1949).

[19]C. G. Baker and H. A. Sober, J. Am. Chem. Soc., *75*, 4058 (1953).

β-ALANINE-3-C^{14}

$$ClCH_2COOK \xrightarrow{KC^*N} NC^*H_2COOK \xrightarrow[NH_3,KOH]{H_2 \atop Ni} NH_2C^*H_2CH_2COOH$$

P. Fritzson and L. Eldjarn, Scand. J. Clin. and Lab. Invest., *4*, 375 (1952); P. Fritzson, Acta Chem. Scand., *9*, 1239 (1955).

A. Procedure (Note 1)

To a stirred solution of 0.1046 g. of potassium cyanide-C^{14} and 0.025 g. of potassium hydroxide in 5 drops of water is added dropwise 1 ml. of aqueous solution containing 3.75 g. of chloroacetic acid and 1.56 g. of anhydrous sodium carbonate (Note 2) diluted to 25 ml. The mixture is heated for 40 minutes on a steam-bath, then cooled, treated with 1.90 ml. of 1.124 *N* hydrochloric acid and evaporated under reduced pressure. The residue is treated with a few ml. of absolute ether and again evaporated. The residue is extracted with 4 portions of absolute ether, and the solution is evaporated to dryness under an air stream in a hydrogenation apparatus (Note 3).

The residue of cyano-C^{14}-acetic acid is treated with one drop of water, and the mixture is adjusted to pH 10–11 with 0.9 ml. of 2 *N* potassium hydroxide. The stirred mixture is treated with 4 ml. of 35% ammonium hydroxide (Note 4) and 0.10 g. of suspended Raney nickel, then hydrogenated for 17 hours at 2 atmospheres and 20° (Note 5). The catalyst is separated by centrifuging, and the combined solution and washings are concentrated to small volume under reduced pressure. The residue is acidified with 0.145 ml. of glacial acetic acid, and the mixture is heated to 50° for a few minutes, then evaporated to dryness. The oil is dissolved in 4–5 drops of water, and the solution is diluted with 5 ml. of

absolute alcohol, seeded, and stored overnight in a refrigerator. The crystalline product is separated from the oily residue by decantation through a filter and recrystallized from water-alcohol to constant specific activity; yield 0.0634 g. (44%), m.p. 196° (Note 6).

B. Notes

1. The procedure is essentially that of Weiner[1] for preparing cyanoacetic acid and is a modification of that of Ruggli[2,3] for the hydrogenation.

2. The excess of acid is sufficient to neutralize 92% of the free alkali used to stabilize the cyanide-C^{14}.

3. A diagram of the simple hydrogenation apparatus is presented by Fritzson and Eldjarn. A small reaction flask containing a magnetic stirring bar is connected with standard taper joints to a gas burette filled with hydrogen over mercury.

4. Ammonia is present to suppress the formation of secondary amines. The free acid, or salt, is more readily reduced, in this case, than is the ester.[3]

5. The absorption of hydrogen is 99.6% of theoretical.

6. Further product is recovered by treating the combined mother liquors with carrier.

C. Other Preparations

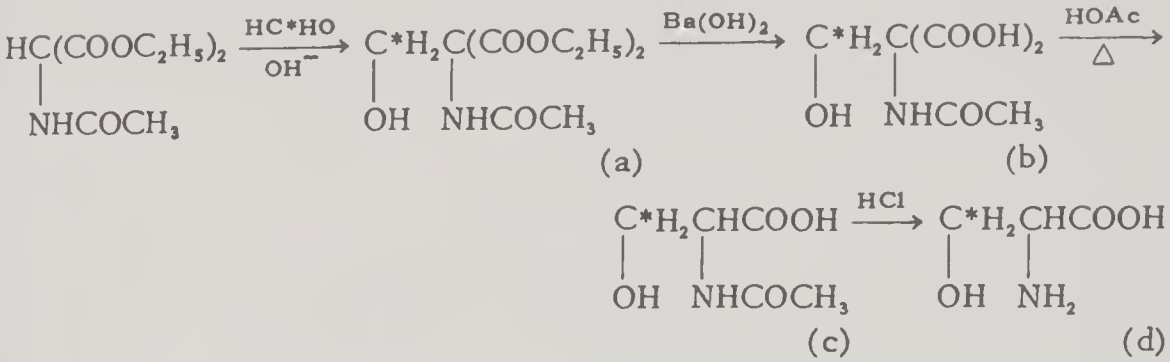
In a similar manner from potassium chloroacetate-1-C^{14} and potassium chloroacetate-2-C^{14}, respectively, are prepared: cyanoacetic-1-C^{14} acid, cyanoacetic-2-C^{14} acid, β-alanine-1-C^{14} and β-alanine-2-C^{14}.

β-Alanine-1-C^{14} has been synthesized by Martignoni[4] from sodium cyanide-C^{14} by the sequence: hydracrylonitrile-1-C^{14}, 3-bromopropionic-1-C^{14} acid, and the final product by the Delépine reaction;[5] over-all yield 19%, m.p. 194–197° (dec.) from water. The radioactivity was recovered quantitatively in a single sharp peak, when the product was chromatographed on a starch column.[6] The product gave a ninhydrin color value[6] identical with that of an authentic sample.

[1] *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 376.
[2] P. Ruggli and A. Businger, U. S. 2,367,436; Chem. Abstracts, 39, 3012 (1945).
[3] P. Ruggli and A. Businger, Helv. Chim. Acta, *25*, 35 (1942).
[4] P. Martignoni and T. Winnick, J. Biol. Chem., *208*, 251 (1954).
[5] N. L. Wendler, J. Am. Chem. Soc., *71*, 375 (1949).
[6] S. Moore and W. H. Stein, J. Biol. Chem., *176*, 367 (1948); *211*, 907 (1954).

SERINE-3-C^{14}
(2-Amino-3-hydroxypropionic-3-C^{14} Acid)

METHOD I



M. Levine and H. Tarver, J. Biol. Chem., *184*, 427 (1950).

A. Procedure (Note 1)

(a) *Ethyl Acetamido(hydroxymethyl-C^{14})malonate.* A mixture of 20 ml. of 0.4% formaldehyde-C^{14} solution (2.9 mmoles), 3.1 mmoles of ethyl acetamidomalonate and two drops of saturated barium hydroxide solution is set aside at room temperature for 24 hours in a stoppered 100-ml. flask (Note 2). The solution is concentrated to 4 ml. under reduced pressure and set aside for 30 hours (Note 3).

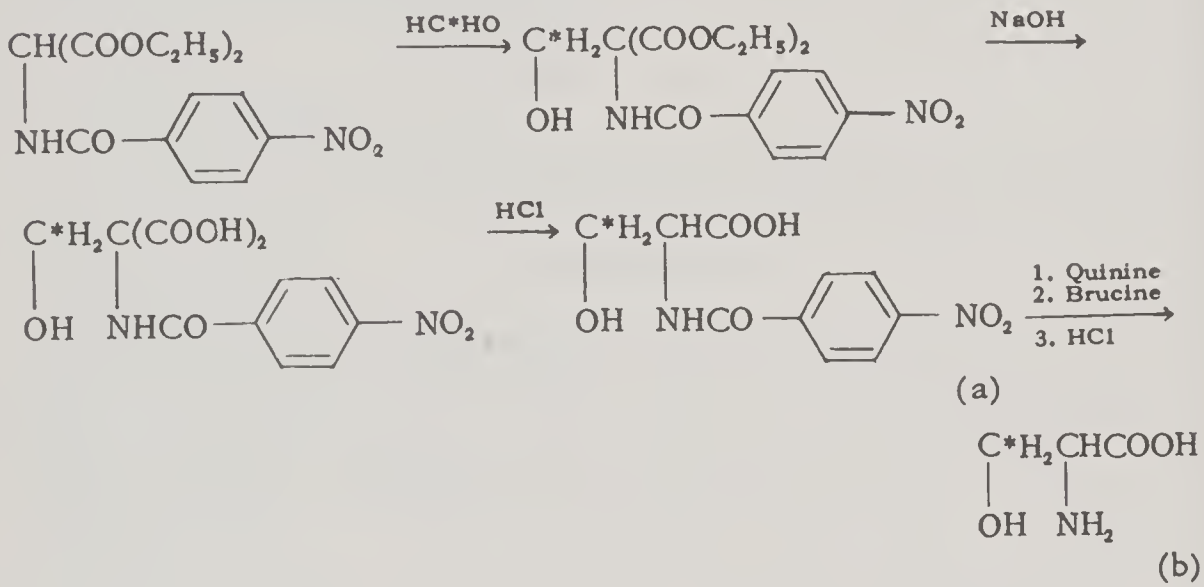
(b) *Acetamido(hydroxymethyl-C^{14})malonic Acid.* To the crude ester is added 1.1 g. of hydrated barium hydroxide (3.5 mmoles); then the mixture is set aside for 12 hours to hydrolyze and finally is acidified with 1 ml. of acetic acid and 15 ml. of water.

(c) N-*Acetylserine-3-C^{14}, (2-Acetamido-3-hydroxypropionic-3-C^{14} Acid).* The substituted malonic acid is decarboxylated quantitatively by refluxing for 1 hour with 1 ml. of acetic acid and 15 ml. of water (Note 3). The clear yellow solution is evaporated to dryness on a water-bath under reduced pressure.

(d) *Serine-3-C^{14}, (3-Hydroxyalanine-3-C^{14}).* The crude *N*-acetylserine-3-C^{14} is taken up with 8 ml. of hot water and refluxed for 1 hour with 20 ml. of 8 *N* hydrochloric acid and 0.5 ml. of 10 *N* sulfuric acid. The mixture is evaporated to dryness on a hot water-bath under reduced pressure, and the process is repeated after the addition of water. The barium is removed by treatment with *N* sulfuric acid and centrifugation. The supernatant liquid is passed through a 2 × 10-cm. column of Duolite A-2, followed by the barium sulfate washings; the eluate (250 ml.) is evaporated to dryness under reduced pressure. The crude product is decolorized in aqueous solution with Norit A, and the filtrate is evaporated to dryness. The colorless residue is dissolved in 6 ml. of hot water, and the solution is diluted with 15 ml. of alcohol and stored overnight. The product is

filtered off, washed with a solution of 2 ml. of water and 5 ml. of ethanol, then recrystallized again and finally dried over phosphorus pentoxide. The yield is 0.160 g., 58% based on formaldehyde. An additional 9% radiochemical yield is recovered (0.161 g.) by recrystallizing 0.170 g. of carrier from the mother liquors and washings (Note 4). Degradation studies indicate that there is no rearrangement (Note 5).

METHOD II



H. R. V. Arnstein, Biochem. J., *49*, 439 (1951); Nature, *164*, 361 (1949).

A. Procedure (Note 6)

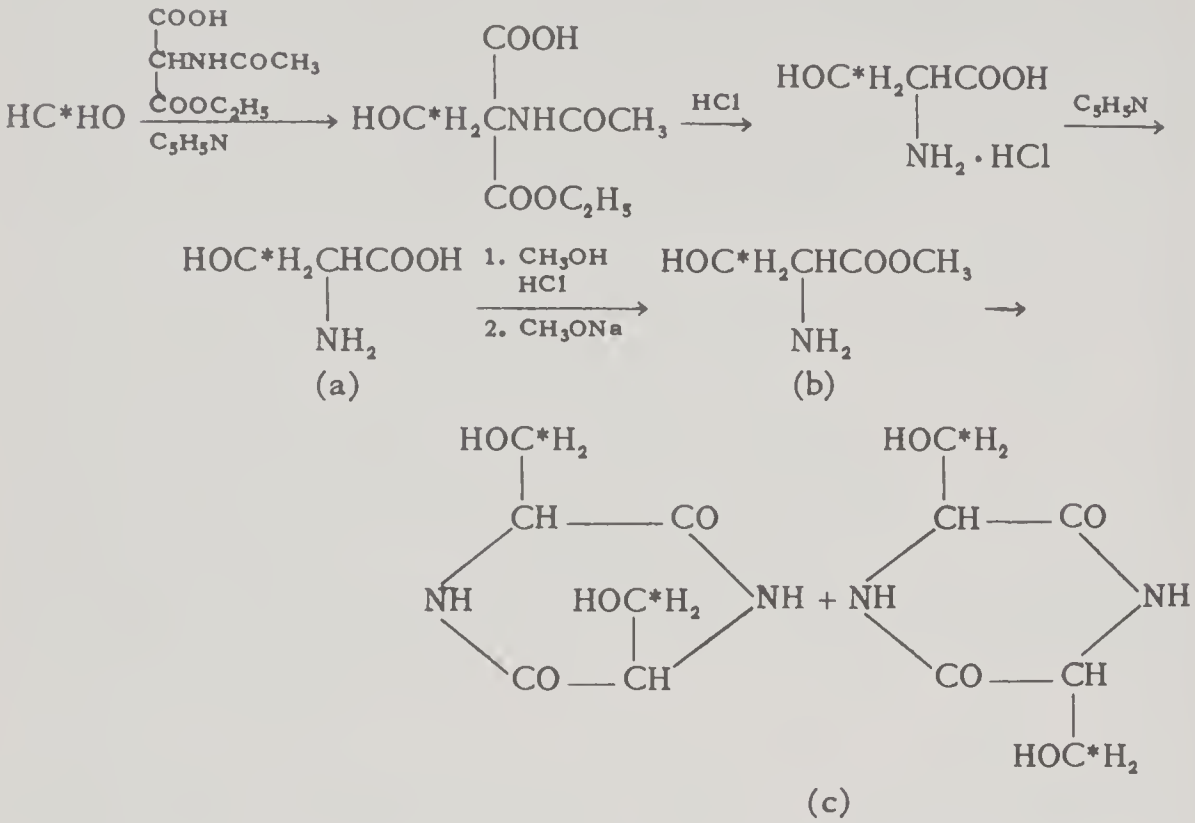
(a) N-p-*Nitrobenzoylserine-3-*C^{14}. A solution of 0.282 g. of formaldehyde-C^{14} (9.6 ml. of aqueous solution), 3.25 g. of ethyl *p*-nitrobenzamidomalonate (Note 7) and 30 ml. of 1 : 1 aqueous-pyridine is shaken for 20 hours. The mixture is evaporated to dryness and the residue (Note 8) is partially hydrolyzed with 2 molar amounts of *N* sodium hydroxide at room temperature, then treated with a like amount of hydrochloric acid and refluxed for 1.5 hours. The mixture is evaporated to dryness, a little water is added, and the evaporation is repeated. The residue is dried under vacuum over potassium hydroxide and then extracted with alcohol. The solution is filtered, treated with a slight excess of pyridine and cooled to crystallize the crude product; yield 1.80 g. (51.5%), m.p. 167–173° (Note 9). Purification is effected by dilution with 5.70 g. of carrier, m.p. 205°, and recrystallization from water; recovery 6.15 g.

(b) *D-Serine-3-*C^{14}. A solution of 6.15 g. of *N-p*-nitrobenzoyl-DL-serine-3-C^{14} and 7.85 g. of quinine in 273 ml. of 1 : 1 aqueous-ethanol is stored for 18 hours at 0° The salt is collected and dried under vacuum

over phosphorus pentoxide; yield 7.64 g. The yield of *N-p*-nitrobenzoyl-D-serine-3-C^{14} quinine salt, after two recrystallizations from 62.5 ml. of the dilute alcohol, is 5.89 g., 84%. The salt is dissolved in 56 ml. of warm 50% aqueous ethanol and treated with 10.03 ml. of *N* sodium hydroxide, and the mixture is cooled in ice at once. After standing for 30 minutes at 0°, the supernatant liquid is decanted and evaporated under vacuum to remove alcohol. The residue is taken up in 20 ml. of water, and the filtered solution is acidified with 10 *N* hydrochloric acid to precipitate the crude *N-p*-nitrobenzoyl-D-serine-3-C^{14}; yield 1.97 g., 64.1%. The acid is refluxed for 5 hours with 50 ml. of 5 *N* hydrochloric acid. The solution is evaporated to dryness under vacuum, 20 ml. of water is added, and the evaporation is repeated. The residue is taken up in 50 ml. of hot water, and the solution is stored overnight at 0°, then filtered and evaporated to dryness. The residue is taken up in 20 ml. of 95% alcohol (v/v), treated with 2 ml. of pyridine in 5 ml. of ethanol and cooled to crystallize the product; yield 0.62 g., 48.3% (Note 10). Degradation studies indicate that there is no isotopic rearrangement (see Method I, Note 5).

(c) *L-Serine-3-*C^{14}. The mother liquors from the preparation of the *N-p*-nitrobenzoyl-D-serine-3-C^{14} quinine salt are evaporated under vacuum. The residue is dissolved in 110 ml. of warm water, then treated with 13.35 ml. of *N* sodium hydroxide, and the mixture is cooled in ice, at once. After standing for several hours at 0°, the solution is filtered, concentrated to 10 ml. and acidified with 5 ml. of 5 *N* hydrochloric acid to precipitate the crude *N-p*-nitrobenzoyl-L-serine-3-C^{14}; yield 2.54 g., 83%. The crude acid and 3.96 g. of brucine are dissolved in 20.3 ml. of water, and the solution is cooled to crystallize the *N-p*-nitrobenzoyl-L-serine-3-C^{14} brucine salt. The yield of salt, twice recrystallized from 12.5 ml. of water, is 4.53 g. The salt is dissolved in 40 ml. of hot water, and the solution is treated with 7.08 ml. of 1.01 *N* sodium hydroxide, then cooled in ice, at once. An additional 20 ml. of water is added and, after standing 18 hours at 0°, the precipitate is filtered off and washed with 100 ml. of water. The filtrate is washed with three 100-ml. portions of ether, then acidified to litmus with 1 ml. of 2 *N* hydrochloric acid and concentrated under vacuum at 60° to about 5 ml. The solution is acidified to Congo red with 2 *N* hydrochloric acid and cooled to precipitate the *N-p*-nitrobenzoyl-L-serine-3-C^{14}; yield 1.59 g. (51.7%). The acid is refluxed for 5 hours with 40 ml. of 3 *N* hydrochloric acid; then the solution is cooled, filtered and evaporated to dryness. The residue is dissolved in 20 ml. of 95% ethanol, then treated with 1 ml. of pyridine in 10 ml. of ethanol and cooled to crystallize the product; yield 0.413 g., 32.5% (Note 11). Degradation studies indicate that there is no isotopic rearrangement.

METHOD III



F. Kögl and A. M. Municio, Hoppe-Seyler's Z. physiol. Chem., *300*, 17 (1955).

A. Procedure

(a) *Serine-3-C^{14}.* To 66.4 ml. of aqueous formaldehyde solution containing 1.104 g. of formaldehyde and approximately 9.7 mmoles of formaldehyde-C^{14} (Note 12) is added a solution containing 7.56 g. of acetamidomalonic acid monoethyl ester (Note 13), 0.2 ml. of twice-distilled pyridine and 40.8 ml. of 0.97 *N* sodium hydroxide. The mixture is stored for 4 days at room temperature; then the excess formaldehyde is removed at 100° with an air stream (Note 14), and the mixture is evaporated to dryness.

The residual crude acetamido(hydroxymethyl-C^{14})malonic acid monoethyl ester is heated with 38 ml. of 1.05 *N* hydrochloric acid for 30 minutes on a water-bath. The mixture is refluxed with 20 ml. of 10 *N* hydrochloric acid for 1.5 hours, then decolorized with charcoal and evaporated under vacuum.

The crude serine-3-C^{14} hydrochloride is dried over potassium hydroxide and extracted into alcohol solution, which is treated with an excess of pyridine and stored in a refrigerator to crystallize the produce; yield 2.87 g., 70%. The presence of only one isotopic compound is indicated by paper chromatography with phenol-water.

(b) *Serine-3-C^{14} Methyl Ester Hydrochloride, (Methyl 2-Amino-3-hydroxypropionate-3-C^{14} Hydrochloride)* (Note 15). Dry hydrogen chloride is

passed into a suspension of 10 g. of serine-3-C^{14} and absolute methanol. The resulting solution is evaporated under vacuum, and the residue is retreated several times in the same manner to complete the esterification. The product is washed with absolute alcohol and ether, then vacuum-dried over potassium hydroxide; yield 12 g. (80%), m.p. 133°.

(c) *3,6-Bis(hydroxymethyl-C^{14})-2,5-piperazinedione*, [cis-(*and* trans-)-*Bis(serine-3-C^{14}) Anhydride*] (Note 15). To a solution of the ester hydrochloride in absolute methanol is added the calculated amount of 2% sodium methylate solution to liberate the free base. The mixture is diluted with ether, stored for 1 hour and filtered to remove sodium chloride. The filtrate is evaporated under vacuum, and the syrupy residue is stored at room temperature for 2 days. The crystalline product is collected and washed with a small amount of methanol and ice water. A second fraction, obtained from the mother liquor by treatment with a drop of water, raises the yield to 3.74 g., 69% (Note 16).

The mixture of *cis*- and *trans*-forms is diluted with 3.13 g. of carrier (prepared in the same manner) and fractionally recrystallized from water, yielding 3.4 g. of the *trans*-form, m.p. 266°, as four-cornered plates. From the mother liquor is obtained the *cis*-isomer in the form of prisms, m.p. 226°.

B. Notes

1. The procedure is a modification of the method described by King,[1] without isolation of the intermediates (which he describes).

2. Arnstein (see Method II) describes the use of pyridine as catalyst for this condensation. He also found that the sodium salt of the half ester condenses with formaldehyde just as readily, eliminating the preliminary alkaline hydrolysis. The yield of serine is 70%.

3. The vapors are passed through a series of scrubbers containing concentrated ammonium hydroxide to trap formaldehyde-C^{14}, some 10–20% being recovered during the various steps of the serine synthesis. The yield of condensation product is nearly quantitative. The direct acid hydrolysis of this compound does not yield serine, but rather the degradation products, pyruvic acid and ammonium chloride.

4. The over-all yield compares well with the isotopic yield of 72% obtained by Elwyn and Sprinson[2] by this method.

5. The C-3 is cleaved by treatment with periodic acid[3] and assayed as the formaldehyde-C^{14}-dimedon derivative, m.p. 191.0–191.5°, which may be determined in the 5 to 25-micromole range by alkaline titration in the presence of phenol red.

6. The procedure is a modification of Method I designed to facilitate the optical resolution by the method of Fischer and Jacobs.[4] An optical

resolution is described by Velluz,[5] and the enzymatic method of resolution has been used by Elwyn.[6,7] A degradation is outlined by Nakada.[8]

7. Directions are given by Arnstein for preparing this starting material, which is used in slight excess, by acylating ethyl aminomalonate in pyridine at 0°.

8. In preliminary runs the condensation product, ethyl (hydroxymethyl-C^{14})-*p*-nitrobenzamidomalonate, was obtained in 94% yield, m.p. 100–102°.

9. The earlier paper reported a yield of 68% based on formaldehyde, m.p. 191–193°. The hydrolysis was done in three steps.

10. After one recrystallization from water-ethanol, $[\alpha]_D^{23}$° + 6.60° ± 0.2° (*c* 10.66 water, 1-dm. tube).

11. After one recrystallization from water-ethanol, $[\alpha]_D^{20}$° − 6.59° ± 0.4° (*c* 7.685 water, 1-dm. tube).

12. The formaldehyde was prepared from methanol-C^{14} (80% yield) which was prepared, in turn, from 2.656 g. of barium carbonate-C^{14}.

13. The monoethyl ester is prepared by hydrolyzing ethyl acetamidomalonate with the calculated amount of *N* sodium hydroxide in 50% alcohol; yield 85%, m.p. 131°.

14. The formaldehyde-C^{14} (0.23 g.) is condensed in a 0.4% aqueous solution of dimedon with the help of Dry Ice-acetone.

15. The procedure is an adaptation of that of Fischer.[9]

16. The product gives a negative reaction with ninhydrin and with bromphenol blue.

C. Other Preparations

Elwyn and Sprinson[2] have prepared L- and D-serine-3-C^{14} in 36% and 43% yield, respectively, from DL-serine-3-C^{14} by essentially the procedure described.

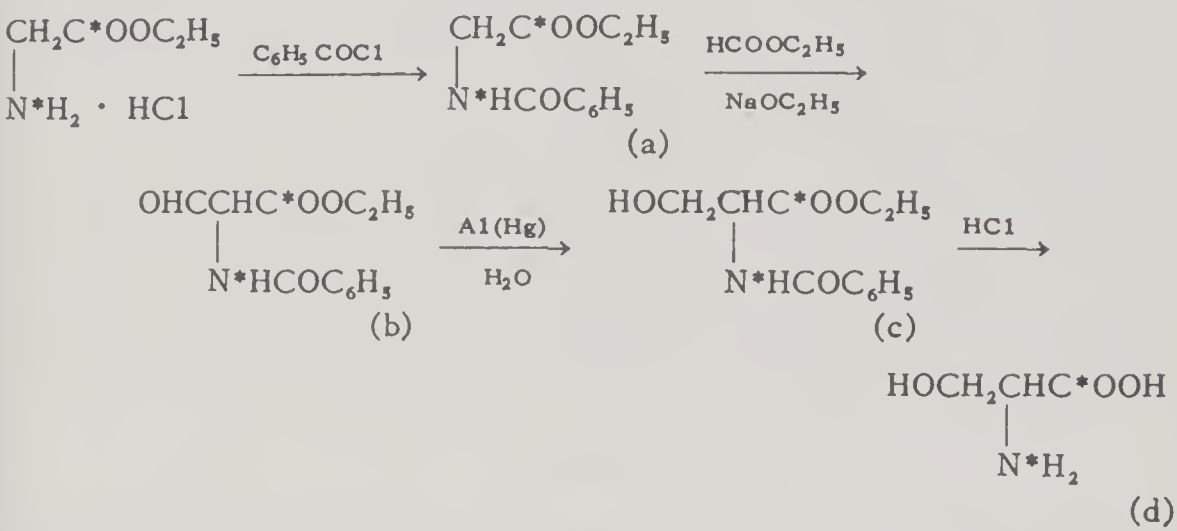
L-Serine-3-$C^{14}_{1/1}$-3-$H^2_{2/1}$ has been prepared[6] (10% yield based on a mixture of 1 mmole of formaldehyde-C^{14} and 0.11 mmole of formaldehyde-H_2^2) by Method I through the sequence: ethyl (hydroxymethyl-$C^{14}_{1/1}$-$H^2_{2/1}$)-malonate, (hydroxymethyl-$C^{14}_{1/1}$-$H^2_{2/1}$)malonic acid, *N*-acetylserine-3-$C^{14}_{1/1}$-3-$H^2_{2/1}$ and serine-3-$C^{14}_{1/1}$-3-$H^2_{2/1}$. The serine was resolved enzymatically[7] through the *N*-chloroacetylserine-3-$C^{14}_{1/1}$-3-$H^2_{2/1}$; see D-alanine-1-C^{14}. As glycine gives trouble during the resolution, its removal is accomplished by chromatography on Dowex-50 with 1.5 *N* hydrochloric acid.

The preparation of L-serine-3-$C^{14}_{1/1}$-3-$H^2_{1/1}$ has been reported;[6] see serine-1-C^{13}-N^{15}. The resolution was accomplished enzymatically (49% yield) by hydrolysis of the *N*-chloroacetyl derivative with Armour acylase.[7]

[1]J. A. King, J. Am. Chem. Soc., 69, 2738 (1947).
[2]D. Elwyn and D. B. Sprinson, J. Biol. Chem., *184*, 465 (1950).
[3]M. W. Rees, Biochem. J., *40*, 632 (1946).

[4]E. Fischer and W. A. Jacobs, Ber., *39*, 2942 (1906).
[5]L. Velluz, G. Amiard and R. Heymes, Bull. soc. chim. France, (5) *21*, 1015 (1954).
[6]D. Elwyn, A. Weissbach, S. S. Henry and D. B. Sprinson, J. Biol. Chem., *213*, 281 (1955).
[7]V. E. Price, J. B. Gilvert and J. P. Greenstein, J. Biol. Chem., *179*, 1169 (1949).
[8]H. I. Nakada, B. Friedmann and S. Weinhouse, J. Biol. Chem., *216*, 583 (1955).
[9]E. Fischer and U. Suzuki, Ber., *38*, 4173 (1905).

SERINE-1-C^{13}-N^{15}



D. Shemin, J. Biol. Chem., *162*, 297 (1946).

A. Procedure

(a)*Ethyl Hippurate-C^{13}-N^{15}*. Glycine-1-C^{13}-N^{15} ethyl ester hydrochloride is benzoylated by the method of Franzen,[1] which follows (Note 1).

A suspension of 20 g. of glycine ethyl ester hydrochloride, 22 g. of benzoyl chloride and 100 ml. of benzene is refluxed until the evolution of hydrogen chloride ceases (16 hours). The solution is filtered and evaporated, and the residue is vacuum-distilled, b.p. 206° (26 mm). The product is crystallized from ether-petroleum ether; yield 28 g. (95%), m.p. 60.5°.

(b) *Ethyl α-Formylhippurate-C^{13}-N^{15}*. The ethyl hippurate-C^{13}-N^{15} is condensed with ethyl formate by the method of Erlenmeyer and Stoop,[2] which follows.

To a cooled solution of 8 g. of sodium in 100–120 ml. of absolute alcohol is added slowly 26 g. of ethyl formate under anhydrous conditions (Note 2). To the suspension of sodium derivative is added 70 g. of ethyl hippurate over a period of 1 hour, with shaking. The mixture sets

to a thick mass and is set aside for 10 days. The sodium salt is separated, washed with absolute alcohol and dissolved in water. Acidification with hydrochloric acid forms an oil, which is extracted into ether. The ether solution is dried and evaporated to give the product as an oil in 80–90% yield (Note 3).

(c) N-*Benzoylserine-1-C*^{13}-*N*^{15} *Ethyl Ester.* The ethyl α-formylhippurate-C^{13}-N^{15} is reduced by the method of Erlenmeyer and Stoop,[2] which follows.

The crude formylhippuric ester is dissolved in 10–15 parts of moist ether and treated with small portions of aluminum amalgam so that there is a continuous gradual evolution of hydrogen (Note 4). The reduction is complete in 4–5 days, as shown by the absence of color when tested with ferric chloride. The ether solution is filtered, dried over magnesium sulfate and distilled, and the product is collected in 60% yield. The product is crystallized from benzene, m.p. 80°.

(d) *Serine-1-C*^{13}-*N*^{15}. The *N*-benzoylserine-1-C^{13}-N^{15} ethyl ester is hydrolyzed by heating under reflux with 20% hydrochloric acid. Most of the acid is removed by evaporation under reduced pressure; the benzoic acid is removed by filtration and the residual chloride by treatment of the filtrate with silver carbonate. The filtered solution is concentrated, and the product is precipitated by dilution with alcohol (Note 5).

B. Notes

1. The starting material is prepared by condensing (chloromethyl)-phthalimide-N^{15} with sodium cyanide-C^{14}, hydrolyzing the (cyano-C^{14}-methyl)phthalimide-N^{15} (see glycine-1-C^{14}, Method II), and then esterifying the acid (see C_1^{13}-tryptophan).

2. Unless the sodium and alcohol are quite pure, decomposition takes place with evolution of carbon monoxide.

3. The ester is crystallized with great difficulty, m.p. 128°.

4. A drop of water is added to the ether from time to time.

5. The *p*-nitrobenzoyl derivative of DL-serine-1-C^{13}-N^{15} is resolved by forming the quinine salt of the D-acid and the brucine salt of the L-acid, according to the classical method of Fischer and Jacobs; see serine-3-C^{14}, Method II.

C. Other Preparations

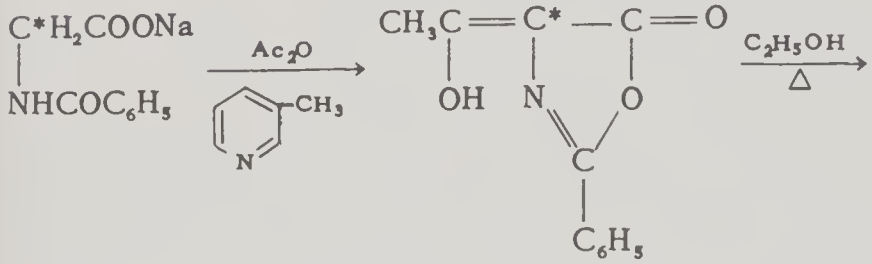
Serine-3-$C^{14}_{1/1}$-3-$H^{2}_{1/1}$ has been prepared[3] from ethyl formate-$C^{14}_{1/1}$-$H^{2}_{1/1}$ (12% yield based on formic acid) through the intermediates ethyl α-formyl-$C^{14}_{1/1}$-$H^{2}_{1/1}$-hippurate and *N*-benzoylserine-3-$C^{14}_{1/1}$-3-$H^{2}_{1/1}$ ethyl ester.

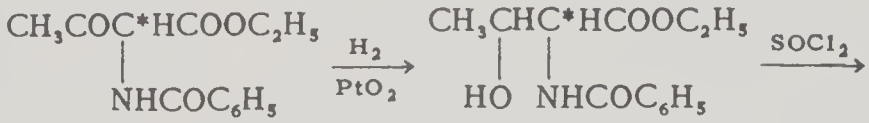
[1]H. Franzen, Ber., *42*, 2465 (1909).

[2]E. Erlenmeyer, Jr. and F. Stoop, Ann., *337*, 236 (1904); E. Erlenmeyer, Jr., Ber., *35*, 3769 (1902).

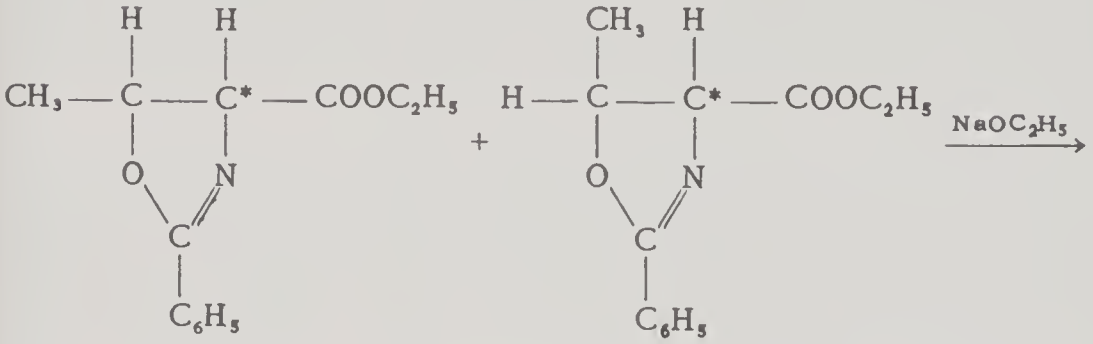
[3]D. Elwyn, A. Weissbach, S. S. Henry and D. B. Sprinson, J. Biol. Chem., *213*, 281 (1955); D. Elwyn and D. B. Sprinson, J. Biol. Chem., *184*, 465 (1950).

L-THREONINE-2-C^{14}
(L-2-Amino-3-hydroxybutyric-2-C^{14} Acid)





(a) (b)



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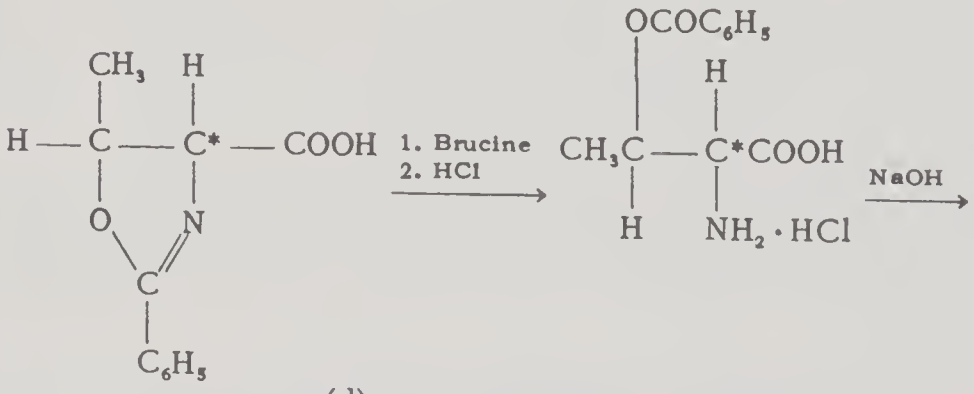
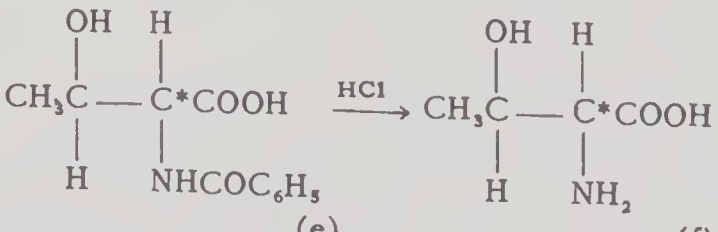


(d)



(e) (f)

A. I. Krasna, P. Peyser and D. B. Sprinson, J. Biol. Chem., *198*, 421 (1952).

A. Procedure

(a) *Ethyl 2-Benzamidoacetoacetate-2-C^{14}.* The hippuric-α-C^{14} acid, prepared from 5.0 g. (67 mmoles) of glycine-2-C^{14}, is converted to an alcoholic solution of the product by an adaptation of the procedure of Attenburrow, Elliott and Penny,[1] which follows (Note 1).

A mixture of 878 g. of sodium hippurate, 1330 ml. of 3-picoline and 1310 ml. of acetic anhydride is mixed for 2 hours in a ball mill. The green solution is separated, and the excess acetic anhydride is decomposed by stirring with 750 ml. of cooled alcohol. The vigorously stirred solution is diluted with 7.5 l. of water, then acidified to Congo red with 6 *N* hydrochloric acid. The insoluble 2-phenyl-4-(1-hydroxyethylidene)-2-oxazolin-5-one is collected and washed with water. The crude material is dissolved in *N* sodium hydroxide solution, then decolorized with charcoal and reprecipitated by acidification, as before; yield 739 g. (83.4%), m.p. 194–195° (dec.). Further purification may be accomplished by chromatography on alumina of the sodium salt in 95% acetone solution. The column is washed with 95% acetone solution and developed with 80% acetone. The oxazolinone forms yellow needles from ethyl acetate, m.p. 198–199° (dec.).

A mixture of 10 g. of the oxazolinone and 100 ml. of absolute alcohol is refluxed for 2 hours, giving a quantitative yield of the desired product.

(b) *Ethyl 2-Benzamido-3-hydroxybutyrate-2-C^{14}* (Note 2). The alcoholic solution of ethyl 2-benzamidoacetoacetate-2-C^{14} is refluxed for 0.5 hour with a little Raney nickel catalyst, to remove traces of sulfur compounds. The filtered solution of ketoester (100 ml.) is hydrogenated over 0.6 g. of platinum oxide at 35 p.s.i. (Note 3). The catalyst is filtered off, the solution is evaporated to dryness, and the residue is dried to constant weight by distillation of added benzene under reduced pressure at the water pump.

(c) *Ethyl 5-Methyl-2-phenyl-2-oxazoline-4-carboxylate-4-C^{14}.* The dry, crude ethyl 2-benzamido-3-hydroxybutyrate-2-C^{14} is cyclized by an adaptation of the method of Elliott,[2] which follows (Note 4). The yield is 8.2 g. (35 mmoles).

To 200 ml. of stirred, ice-cooled, purified thionyl chloride is added slowly a solution of 200 g. of 2-benzamido-3-hydroxybutyrate in 100 ml. of chloroform over a period of 1.5 hours, while the temperature is maintained at 5–6°. The mixture is stored at 0° for 16 hours, then concentrated under vacuum at 35–38°. The residual syrup is poured, without delay, into a solution of 170 g. of anhydrous sodium carbonate and 1600 ml. of water. The emulsion is extracted with four portions of petroleum ether (2500 ml. total), and the combined extracts are washed with water,

dried and evaporated to dryness. The yield of *cis*- and *trans*-oxazolines is 175 g.

(d) trans-*5-Methyl-2-phenyl-2-oxazoline-4-carboxylic-4-C*^{14} *Acid* (Note 5). The crude, oily mixture of *cis*- and *trans*-oxazolines is dissolved in 40 ml. of absolute ethanol and treated with a solution of 0.8 g. (35 mmoles) of sodium in 40 ml. of absolute ethanol. After standing at room temperature for 5 minutes, the mixture is diluted with 80 ml. of water and refluxed for 15 minutes to hydrolyze the *trans*-ester (Note 6). The solution is concentrated under vacuum to 35 ml., then cooled to 0°, and the pH is adjusted to 3.5 by addition of 5 *N* hydrochloric acid dropwise with stirring and maintenance of the temperature. The supernatant liquid is decanted and kept cold, while the oil is treated with a little ether to induce crystallization. The solid is collected, washed with a small amount of ice-water and dried; yield 0.99 g., m.p. 137–142°. Another crop of crystals, m.p. 139–142°, is obtained by treating the supernatant solution with a small amount of ether, making the total yield 3.2 g. (16 mmoles).

(e) N-*Benzoyl-L-threonine-2-C*^{14}. The DL-*trans*-carboxylic acid is dissolved with warming in a solution of 6.1 g. of anhydrous brucine and 12 ml. of absolute ethanol (Note 7). The filtered solution is evaporated to dryness under vacuum, and the residue is dissolved in a mixture of 1.8 ml. of absolute ethanol and 8.8 ml. of anhydrous ethyl acetate and set aside to crystallize (Note 7). The salt is collected, washed with a small amount of cold ethyl acetate and recrystallized twice from ethyl acetate (Note 8). The yield of *trans*-L-5-methyl-2-phenyl-2-oxazoline-4-carboxylic-4-C^{14} acid brucine salt is 3.0 g., m.p. 126–129°. The salt is decomposed by dissolution in *N* sodium hydroxide and extraction with chloroform. The aqueous solution is adjusted to pH 1 with 5 *N* hydrochloric acid, with resultant ring fission of the oxazolinecarboxylic acid to *O*-benzoyl-L-threonine-2-C^{14}. After 4 hours at room temperature, the stirred mixture is made basic to phenolphthalein with sodium hydroxide, then acidic to Congo red with hydrochloric acid, and the crystalline *N*-benzoyl-L-threonine-2-C^{14} is collected; yield 0.99 g., m.p. 140–141° (Note 9).

(f) *L-Threonine-2-C*^{14}. *N*-Benzoyl-L-threonine-2-C^{14} (0.530 g.) is hydrolyzed by the method of Elliott,[3] which follows. The yield is 0.215 g., 10% based on glycine, $[\alpha]_D^{27}$-28.5° (2.7% in water).

A mixture of 2 g. of *N*-benzoylthreonine and 20 ml. of 6 *N* hydrochloric acid is refluxed for 5 hours, cooled and extracted with ether to remove benzoic acid. The aqueous solution is evaporated to dryness under reduced pressure, and the residue is taken up in 20 ml. of hot ethanol, treated with an excess of pyridine and cooled to crystallize the product; yield 0.95 g. (89%), m.p. 264° (dec.). The product is recrystallized from 80% ethanol in the form of hexagonal plates.

B. Notes

1. Both the acetic anhydride and the 3-picoline are redistilled, the latter after drying over potassium hydroxide.

2. The procedure is according to the method of Pfister[4] as modified by Meltzer and Sprinson.[5]

3. At the completion of the reaction, a red color is no longer observed with ferric chloride.

4. The product is a mixture of the *cis*- and *trans*-forms. Alternative procedures are described by Pfister[4] and by Meltzer and Sprinson;[5] see L-threonine-4-$C^{14}_{1/1}$-$N^{15}_{1/1}$. Thionyl chloride is purified by distillation alternately with 2% by weight of quinoline and yellow beeswax until a colorless product results.

5. The procedure is a modification of the method of Elliott.[3]

6. Alkaline hydrolysis of the *cis*- and *trans*-oxazolines gives only the DL-*trans*-acid.

7. The D-acid does not form a crystalline salt with brucine. When crystallization would not take place, 0.230 g. of insoluble material was removed, m.p. 134–136°.

8. The filtrates are saved for recovery of *N*-benzoyl-DL-threonine-2-C^{14}. The four stereoisomeric 2-amino-3-hydroxybutyric acids are interconvertible.[3]

9. *N*-Benzoylthreonine (m.p. 145–146°) has been prepared[3] in 63.6% yield based on 200 g. of ethyl 2-benzamido-3-hydroxybutyrate, without purification of intermediates. A degradation of threonine is outlined by Wang.[6]

[1]J. Attenburrow, D. F. Elliott and G. F. Penny, J. Chem. Soc., *1948*, 310.

[2]D. F. Elliott, J. Chem. Soc., *1949*, 589.

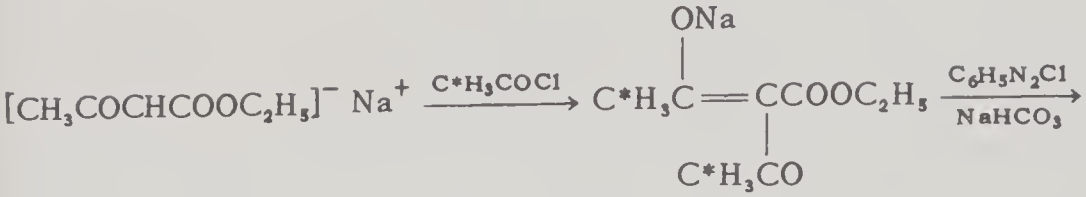
[3]D. F. Elliott, J. Chem. Soc., *1950*, 62.

[4]K. Pfister, 3rd., C. A. Robinson, A. C. Shabica and M. Tishler, J. Am. Chem. Soc., *71*, 1101 (1949).

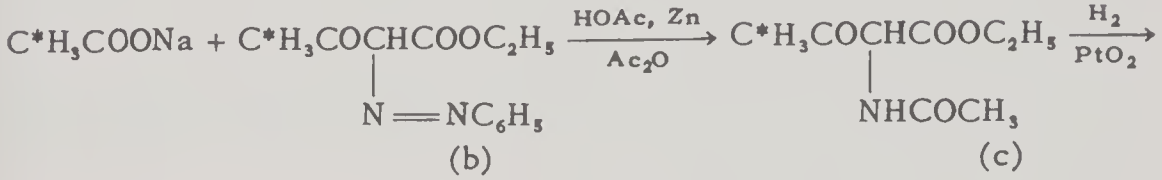
[5]H. L. Meltzer and D. B. Sprinson, J. Biol. Chem., *197*, 461 (1952).

[6]C. H. Wang, B. E. Christensen and V. H. Cheldelin, J. Biol. Chem., *213*, 365 (1955).

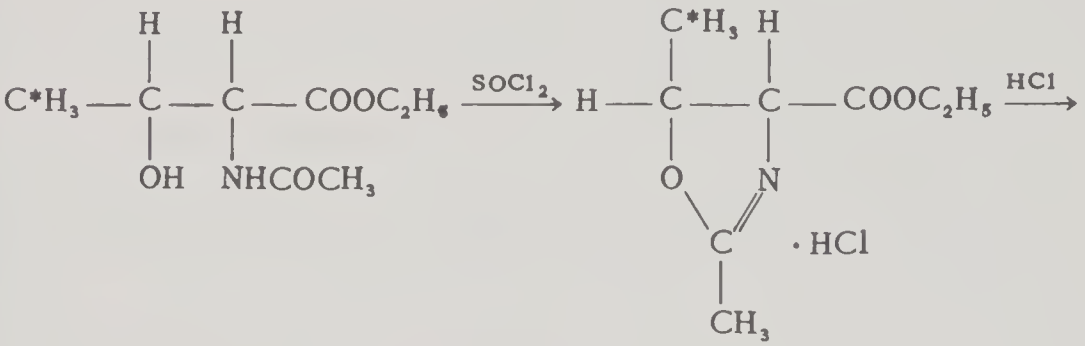
THREONINE-4-C^{14}



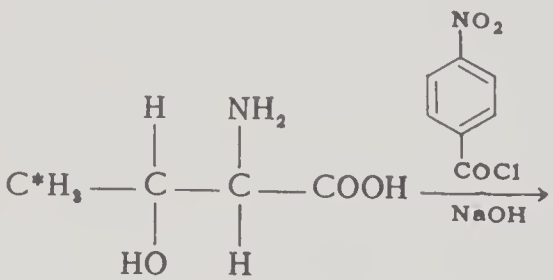
(a)



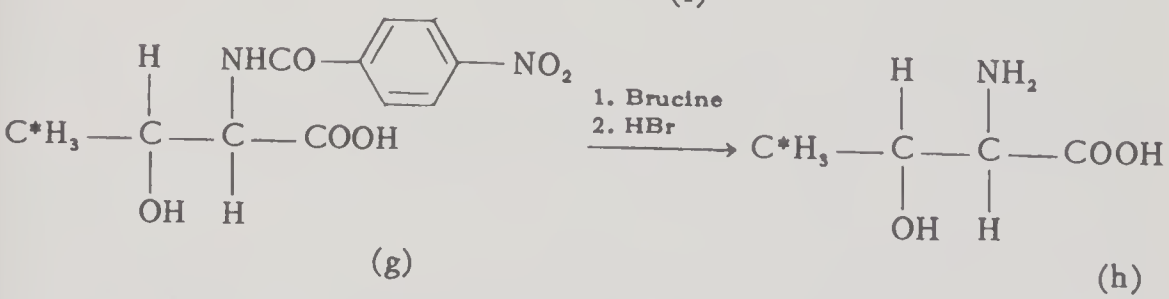
(b) (c)



(d) (e)



(f)



(g) (h)

H. L. Meltzer and D. B. Sprinson, J. Biol. Chem., *197*, 461 (1952).

A. Procedure (Note 1)

(a) *Ethyl 2-Acetyl-2-C^{14}-sodiumacetoacetate-4-C^{14}* (Note 2). A solution of acetyl-2-C^{14} chloride, prepared from 25 mmoles of sodium acetate-2-C^{14} (Note 3), in 35 ml. of absolute ether is added dropwise to a stirred suspension of 6.9 g. (45.3 mmoles) of ethyl sodiumacetoacetate in 100 ml. of

absolute ether at −7°. The precipitated product (Note 4) is filtered off and washed with ether; the yield is good.

(b) *Ethyl 2-Phenylazoacetoacetate-4-C^{14}* (Note 5). To a stirred mixture of the ethyl 2-acetyl-2-C^{14}-sodiumacetoacetate-4-C^{14}, 160 ml. of absolute ethanol, 15 g. of sodium bicarbonate and 30 ml. of water, is added at 10°, over a 20-minute period, a solution of benzenediazonium chloride[1] prepared from 2.5 ml. of aniline. The solution is stirred for 20 minutes at 10°, then cooled, and diluted at intervals with 25-ml. portions of cold water until no further precipitation occurs. The product is collected, and the mother liquor is acidified with sulfuric acid and steam-distilled into alkali to recover sodium acetate-2-C^{14} (14.5 mmoles), which is then converted into (b), as above.

(c) *Ethyl 2-Acetamidoacetoacetate-4-C^{14}*. The reductive acetylation of ethyl 2-phenylazoacetoacetate-4-C^{14} is performed according to the procedure of Pfister,[2] which follows.

To a vigorously stirred mixture of 513 ml. of glacial acetic acid, 257 ml. of acetic anhydride and 390 g. of zinc dust, maintained at 10–15°, is added slowly over 1–1.5 hours a solution of 217.4 g. (0.927 mole) of ethyl 2-phenylazoacetoacetate in 232 ml. of acetic acid. The mixture is stirred for 3 hours at 25–30°, then filtered, and the salts are washed with glacial acetic acid. The filtrate and washings are combined, concentrated under reduced pressure and stirred with 500 ml. of water at 55° for 5 minutes. The mixture is cooled to 10°, the residual acid is neutralized with dry sodium bicarbonate, and the mixture is stored at 0° for 30 minutes and then filtered to remove acetanilide (113.5 g., 91%). The crude product is extracted into four 250-ml. portions of chloroform, and the combined extracts are evaporated to dryness under reduced pressure. The residue crystallizes upon standing; yield 164.2 g. (94.6%), m.p. 39–42° (Note 6).

(d) N-*Acetylallothreonine-4-C^{14} Ethyl Ester*, (*Ethyl 2-Acetamido-3-hydroxybutyrate-4-C^{14}*). (Note 7). Ethyl 2-acetamidoacetoacetate-4-C^{14} is hydrogenated according to the method of Pfister;[2] which follows.

A solution of 65.5 g. (0.35 mole) of ethyl 2-acetamidoacetoacetate in 525 ml. of water is made just alkaline with 5 ml. of *N* sodium hydroxide, then hydrogenated at 25° and 30–40 p.s.i. over 0.65 g. of platinum oxide. The theoretical amount of hydrogen is absorbed in 4 hours (Note 8). The solution is filtered and concentrated to dryness at the aspirator; then the residue is dissolved in benzene and reconcentrated to constant weight. The yield of thick colorless oil is 32.2 g. (Note 9).

(e) *Ethyl 2-Methyl-5-methyl-C^{14}-2-oxazoline-4-carboxylate Hydrochloride*. The crude ethyl 2-acetamido-3-hydroxybutyrate-4-C^{14} is cyclized by the procedure of Pfister,[2] which follows (Note 10).

A solution of 33.2 g. of crude ethyl 2-acetamido-3-hydroxybutyrate in 40 ml. of anhydrous benzene is treated dropwise at 10–15° with 25.4 ml.

of thionyl chloride. The mixture is stirred for 1 hour without cooling, then poured into 965 ml. of absolute ether and stored in a refrigerator for 16 hours. The crystalline product is collected, washed with dry ether and dried under vacuum over sulfuric acid; yield 31.0 g., m.p. 95–98° (Note 11).

(f) *Threonine-4-C^{14}*, (*2-Amino-3-hydroxybutyric-4-C^{14} Acid*) (Note 12). A mixture of threonine-4-C^{14} and allothreonine-4-C^{14} in the proportion of 4:1 is obtained by hydrolysis of the crude ethyl 2-methyl-5-methyl-C^{14}-2-oxazoline-4-carboxylate hydrochloride according to the following procedure of Pfister.[2] The free amino acids are obtained by passage of the hydrochloride salts through a column of Duolite A-4. The yield is 1.4 g. from 25 mmoles of sodium acetate-2-C^{14}.

The benzene solution of the oxazoline hydrochloride, without isolation of the intermediate, is chilled and treated with cold water, and the benzene layer is separated and extracted with two portions of water. The combined aqueous solutions are washed with 5 ml. of benzene, then refluxed for 2.5 hours, decolorized with charcoal and evaporated to dryness under reduced pressure. The yield of crude threonine is 88.7% based on ethyl 2-acetamidoacetoacetate; the product consists of 83.2% *trans-*, or threonine, configuration determined by bioassay.

(g) *Threonine-4-$C^{14}_{1/1}$-$N^{15}_{1/1}$*. The 1.4 g. of crude threonine-4-C^{14} is mixed with 5.1 g. of a similar crude threonine-N^{15}-allothreonine-N^{15} preparation and purified according to the procedure of Pfister,[2] which follows.

For elimination of allothreonine, 15.6 g. (0.131 mole) of crude threonine (Note 13) is refluxed for 5 minutes with a solution of 3.02 g. (0.131 mole) of sodium in 60.4 ml. of absolute alcohol. The mixture is stored for 16 hours at room temperature; then the insoluble sodium salt of threonine is collected, washed with 15 ml. of alcohol and dried at 50–55°; yield 15.43 g. (83.5%). The sodium salt (15.00 g., 0.106 mole) is treated with 18.5 ml. (0.222 mole) of ice-cold concentrated hydrochloric acid and 120 ml. of isopropyl alcohol. The stirred mixture is heated to 60° and stored in a refrigerator for 12 hours. The sodium chloride is filtered off, and the filtrate is treated with 20.4 ml. of aniline and stored at 5° for 16 hours. The product yield is 11.5 g. (91%). Recrystallization is effected (96.8% recovery) by dissolution of the product in 28.8 ml. of hot water, dilution with 86 ml. of warm ethanol and cooling for 12 hours. Purity is 100% by microbial assay.

(h) *L-Threonine-4-$C^{14}_{1/1}$-$N^{15}_{1/1}$*. The purified DL-threonine-4-$C^{14}_{1/1}$-$N^{15}_{1/1}$ is resolved by the method of Zambito, Peretz and Howe,[3] which follows. The yield of chromatographically pure free acid, obtained by passing the hydrobromide salt through a column of Duolite A-4, is 0.92 g., $[\alpha]^{23}_{D}$-28.2°.

To a stirred, ice-cooled solution of 32 g. (0.269 mole) of DL-threonine, 1200 ml. of water and 270 ml. of *N* sodium hydroxide are added simultaneously, over a period of 1 hour, 50 g. (0.269 mole) of freshly distilled

p-nitrobenzoyl chloride and 135 ml. of 2 *N* sodium hydroxide. The mixture is stirred for 20 minutes, then acidified with 40 ml. of concentrated hydrochloric acid (Congo red) and cooled at 0° for 1 hour. The crude *N*-*p*-nitrobenzoyl-DL-threonine [-4-C^{14}_{1}-N^{15}_{1}] is collected and extracted with 300 ml. of boiling water, to remove 4.1 g. of insoluble *p*-nitrobenzoic acid. The hot solution is cooled to 0–5° for 1 hour; then the crude *N*-*p*-nitrobenzoyl-DL-threonine [-4-C^{14}_{1}-N^{15}_{1}] is filtered off, washed with two 75-ml. portions of ice water, then dried at 60° and extracted twice with warm ether, to remove additional *p*-nitrobenzoic acid. The yield is 50.7 g. (70.5%), m.p. 166–167°.

A warm solution of 64 g. (0.149 mole) of brucine and 40 g. (0.149 mole) of *p*-nitrobenzoyl-DL-threonine [-4-C^{14}_{1}-N^{15}_{1}] in 160 ml. of methanol (50–55°) is cooled to 25°in an ice-bath with stirring and scratching for 5 minutes (Note 14). The stirred mixture is reheated to 50° and again cooled to 25° over a 5-minute period. The precipitate is transferred to suction filter with the mother liquor, then is pulverized in a mortar and washed with two 60-ml. portions of cold methanol and two 50-ml. portions of ether (Note 15). The yield of nearly colorless *N*-*p*-nitrobenzoyl-L-threonine [-4-C^{14}_{1}-N^{15}_{1}] brucine salt is 46 g. (88%), m.p. 145–150° (Note 16). By concentrating the mother liquor, an additional 2 g. (m.p. 147–150°) is obtained, making the combined yield 92%.

To a stirred mixture of 48 g. of the brucine salt of *N*-*p*-nitrobenzoyl-L-threonine [-4-C^{14}_{1}-N_{1}] and 500 ml. of water at 40–50° is added slowly 80 ml. of *N* sodium hydroxide. The mixture is stirred for 1 hour at that temperature, then cooled in an ice-bath for 30 minutes. The precipitate is filtered off and washed with two 100-ml. portions of ice water. Residual traces of brucine are removed from the combined filtrate and washings by extraction with 150 ml. of chloroform and 150 ml. of ether. The aqueous solution is concentrated under reduced pressure to 80 ml., then refluxed for 4 hours with 56 ml. of constant-boiling hydrobromic acid and stored overnight in a refrigerator. The crystallized *p*-nitrobenzoic acid is removed, and the filtrate is evaporated under vacuum. The residue is treated with 50 ml. of water and again evaporated. The residual syrup is separated from sodium bromide by extraction with hot absolute ethanol, followed by evaporation of the solvent. The residue of L-threonine [-4-C^{14}_{1}-N^{15}_{1}] hydrobromide yields 6.25 g. of 99.7 ± 0.1% L-threonine [-4-C^{14}_{1}-N^{15}_{1}] (49.5% based on DL-threonine), $[\alpha]_D^{26}$-27.9° (5% aqueous solution).

Pure D-threonine-4-C^{14}_{1}-N^{15}_{1} is obtained in 43.6% yield from DL-threonine-4-C^{14}_{1}-N^{15}_{1} by a similar procedure.

B. Notes

1. The procedure is a modification of the method of Pfister,[2] the requisite ethyl 2-phenylazoacetoacetate being prepared from ethyl 2-acetyl-

acetoacetate rather than from ethyl acetoacetate. The former compound, labeled in the 4-position, is prepared in higher yield than is ethyl acetoacetate-4-C^{14}.

2. The procedure is according to Michael and Carlson.[4]

3. The acetyl halide is prepared in 75–90% yield by treating the salt with a mixture of benzoic acid and benzoyl halide, according to the procedure of Anker,[5] and then collecting the product distilling at 43–56°.

4. Michael and Carlson[4] stir the cold addition mixture for 0.5 to 1.5 hours and allow the precipitate to settle for 1–3 hours.

5. The procedure is adapted from the method of Bulow and Hailer.[6] The molar specific activity is one-half that of the starting acetyl-2-C^{14} chloride, but considerable acetate-2-C^{14} is recoverable from the reaction mixture. The procedure offers a general method for preparing labeled acetoacetic ester (see ethyl acetoacetate-3-C^{14}), or other β-keto esters,[7] starting from mixed diacyl acetates.

6. Recrystallization from toluene raises the melting point to 47.5–48.5°. The product may also be prepared by catalytic hydrogenation[2] of the starting material, in acetic anhydride-acetic acid, over palladium at room temperature and 25–40 p.s.i.

7. Catalytic hydrogenation of ethyl 2-acetamidoacetoacetate yields a mixture of the *N*-acetyl esters of the two diasterioisomers, allothreonine and threonine, with the former predominating[1] about 4 : 1.

8. A ferric chloride test is negative.

9. Repeated recrystallization from ethyl acetate yields the pure *N*-acetylallorthreonine ethyl ester as prisms melting at 76–77°.

10. The reaction of α-acylamido-β-hydroxy compounds with dehydrating agents to form oxazolines is general, provided any carboxyl group is masked to prevent azlactonization, and is of value as a general method for the interconversion of diasterioisomers containing an amino and hydroxyl group on adjacent asymmetric carbon atoms. The inversion appears to involve the β-carbon atom during ring closure.[8] Several other methods of forming the oxazolines are described by Elliott.

11. Purification, by precipitation with dry ether from a dry chloroform solution, yields material melting at 105–106°. The compound hydrolyzes readily. Exposure to the air for 24 hours froms *O*-acetylthreonine ethyl ester hydrochloride, m.p. 130–131°.

12. The crude product is threonine-4-C^{14} and allothreonine-4-C^{14} in the ratio of 4 : 1. Purification is effected through the alcohol-insoluble sodium salt (see below). Pure threonine, m.p. 229–230°, is obtained in 94.6% yield from the purified *N*-acetylallothreonine ethyl ester.

13. The crude threonine is obtained without purification of the hydrogenation product. A chromatographic isomeric separation has been developed by Shulgin; see threonine-1,2-C_2^{14}.

14. The brucine salt of *N-p*-nitrobenzoyl-L-threonine is a nearly colorless, highly insoluble compound. The salt of the D-isomer exists in two forms, an unstable, low-melting, deep orange-colored, soluble form which slowly changes to a high-melting, light yellow, insoluble product. Time and temperature are, therefore, critical in this method of resolution.

15. The washes are collected in a separate receiver.

16. The salt crystallizes as a dihydrate. The combined filtrate and methanol washes, upon standing overnight, yield 46 g. (88.5%) of the yellow brucine salt of *N-p*-nitrobenzoyl-D-threonine.

[1]C. Bulow and P. Neber, Ber., *45*, 3732 (1912); Chem. Abstracts, 7, 1192 (1913).

[2]K. Pfister, 3rd, C. A. Robinson, A. C. Shabica and M. Tishler, J. Am. Chem. Soc., *71*, 1101 (1949).

[3]A. J. Zambito, W. L. Peretz and E. E. Howe, J. Am. Chem. Soc., *71*, 2541 (1949).

[4]A. Michael and G. H. Carlson, J. Am. Chem. Soc., 57, 165 (1935).

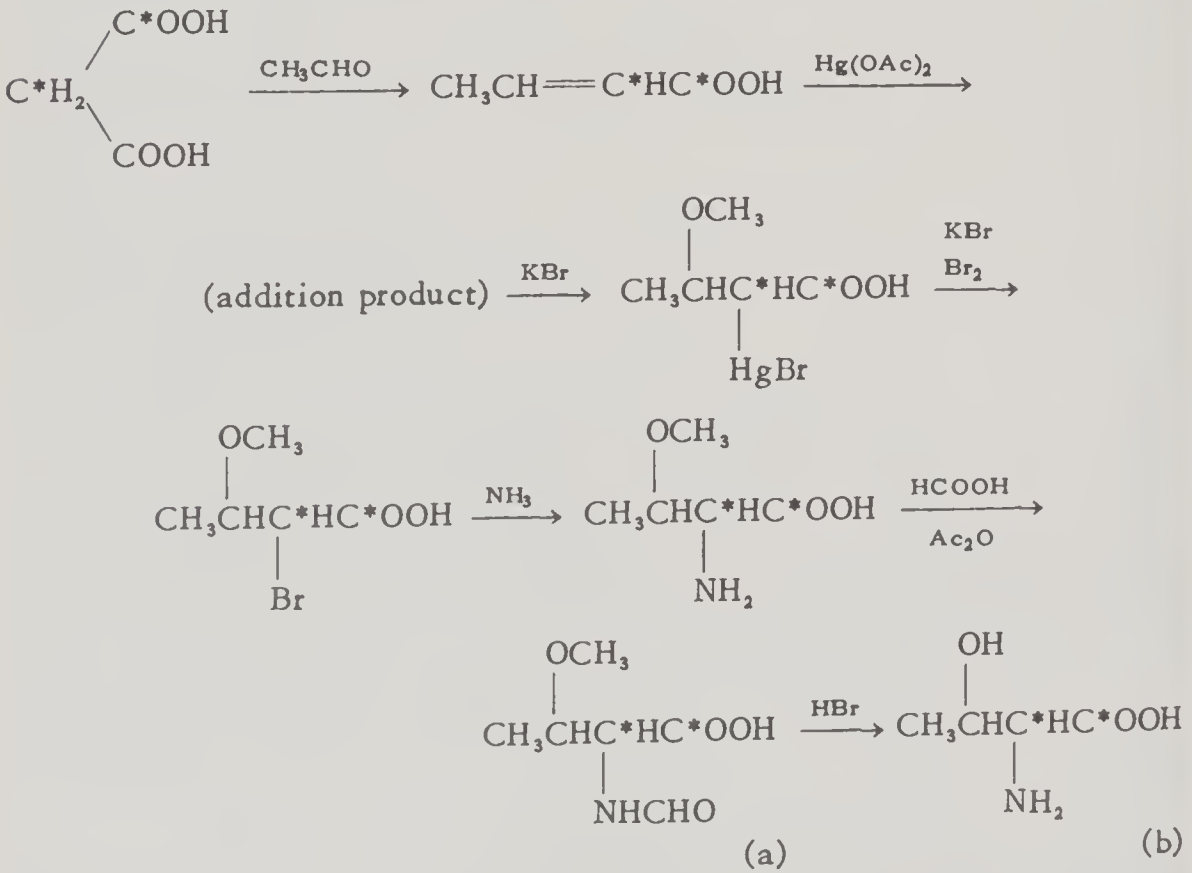
[5]H. S. Anker, J. Biol. Chem., *176*, 1333 (1948).

[6]C. Bulow and E. Hailer, Ber., *35*, 915 (1902).

[7]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 266.

[8]D. F. Elliott, Nature, *162*, 657 (1948); J. Attenburrow, D. F. Elliott and G. F. Penny, J. Chem. Soc., *1948*, 310.

THREONINE-1,2-C_2^{14}



A. T. Shulgin, O. G. Lien, Jr., E. M. Gal and D. M. Greenberg, J. Am. Chem. Soc., *74*, 2427 (1952).

A. Procedure (Note 1)

(a) *2-Formamido-3-methoxybutyric-1,2-C_2^{14} Acid,* (*N-Formyl-O-methylthreonine-1,2-C_2^{14}*). Malonic-1,2-C_2^{14} acid (3.6 g.) is converted to crotonic-1,2-C_2^{14} acid in 84% yield (Note 2). An addition product of unknown structure, formed in nearly quantitative yield by treating the crotonic acid with mercuric acetate in methanol solution, is brominated by treating with aqueous potassium bromide under illumination. The mercury is then exchanged for bromine by treatment with a solution of bromine, potassium bromide, and water, forming the crude diasterioisomeric bromo-acids (Note 3). Amination is effected by heating at 100° with concentrated ammonium hydroxide in a pressure vessel. The mixture of isomeric amino acids is formylated with 85–90% formic acid in the presence of acetic anhydride, and the mixture is evaporated to dryness under reduced pressure. A single crystallization from water gives a product melting at 140–158° (sintering at 136°) (Note 4). Recrystallization from water raises the melting point to 164–170° (sintering at 147°) (Note 5).

(b) *Threonine-1,2C_2^{14}*. The crude *N*-formyl-*O*-methylthreonine-1,2-C_2^{14} is hydrolyzed by refluxing for 2 hours with constant-boiling hydrobromic acid. The mixture is evaporated under reduced pressure, then re-evaporated following dissolution in water. The residue is taken up in absolute alcohol and treated with excess concentrated ammonium hydroxide, and the mixture is stored overnight in a refrigerator. The crude product is collected and recrystallized from water-alcohol; yield 0.443 g., m.p. 232–234°, purity 53%, determined by microbial assay of proven efficiency (Note 6).

(c) *Allothreonine-1,2-C_2^{14}*. The *cis*-isomer is prepared and isolated from the water-soluble filtrate remaining from the crystallization of *N*-formyl-*O*-methylthreonine-1,2-C_2^{14}. The *N*-formyl-*O*-methylallothreonine-1,2-C_2^{14} is worked up in the same manner as above; yield 0.294 g., m.p. 239–241°.

Both isomers are separated quantitatively by chromatography on an 8-ft. × 12-mm. column of Dowex-50 (250/500 mesh) cation exchange resin in the acid form (Note 7). Samples of 0.5 g. in 6 ml. of water are put on the column under 18-cm. pressure, followed by 5-ml. washings of water under pressure. Elution is with 1.5 *N* hydrochloric acid under pressure, at a flow rate of 5 ml. per hour. Peaks of radioactivity are located by a process of automatic sampling and by counting periodic samples.

B. Notes

1. The procedure is a suitable adaptation of that of Carter and West,[1] which was chosen for purposes of evaluating a chromatographic separa-

tion of the two diasterioisomers. The yield of threonine[2] rises from 30–33% to 52–55% if isocrotonic acid is used in place of crotonic acid in the method of West, Krummel and Carter.[3] From a purely preparative standpoint, the preparation of allothreonine[4] with the interconversion[5] to threonine is probably most suitable.

2. No details of this preparation are given. The yield is corrected for a purity of 91%, as determined by titration and comparison with an authentic sample. A preparation by the condensation of acetaldehyde with malonic acid in the presence of pyridine is described by Florence[6] and by v. Auwers.[7]

3. The yield is 88–93% based on crotonic acid at the 2-mole level.[1]

4. *N*-Formyl-*O*-methylthreonine, m.p. 173–174°; *N*-formyl-*O*-methylallothreonine, m.p. 152–153°. As the former isomer is much the less water-soluble, this is the basis for a crude separation.

5. The recrystallized product yields threonine containing much less allothreonine, but the over-all yield is reduced by 50% at this scale. Neither this chemical method nor paper chromatography completely separates the isomers on a small scale. Such mixtures can be separated by fractionation of the sodium salts[5] with ethanol, or by chromatography on a cation exchange resin (see below).

6. Directions are given by Delluva[8] for degrading C^{14}-threonine by periodate oxidation, forming acetaldehyde (C-3,4), formic acid (C-2), and carbon dioxide (C-1). The acetaldehyde is further degraded *via* barium carbonate (C-3) and acetone (C-4,4,3).

7. Directions are given by Shulgin for preparing the column.

C. Other Preparations

The preparation of allothreonine-1-C^{14} has been outlined by Arnstein.[9] 2-Bromobutyric-1-C^{14} acid, prepared with bromine and red phosphorous, was esterified, and the isobutyl 2-bromobutyrate-1-C^{14} was dehydrohalogenated with diethylaniline, forming isobutyl crotonate-1-C^{14}.

Saponification and treatment of the crotonic-1-C^{14} acid with bromine in water gave 2-bromo-3-hydroxybutyric-1-C^{14} acid, which was aminated by the method of Carter.[4]

[1] *Organic Syntheses*, Vol. 20, Wiley, New York, 1940, p. 101.

[2] K. Pfister, 3rd., E. E. Howe, C. A. Robinson, A. C. Shabica, E. W. Pietrusza and M. Tishler, J. Am. Chem. Soc., *71*, 1096 (1949).

[3] H. D. West, G. S. Krummel and H. E. Carter, J. Biol. Chem., *122*, 605 (1938).

[4] H. E. Carter and C. L. Zirkle, J. Biol. Chem., *178*, 709 (1949).

[5] K. Pfister, 3rd., C. A. Robinson, A. C. Shabica and M. Tishler, J. Am. Chem. Soc., *71*, 1101 (1949).

[6] G. Florence, Bull. soc. chim., France, *41*, 440 (1927).

[7] K. v. Auwers, Ann., *432*, 46 (1923).

[8]A. M. Delluva, Arch. Biochem., *45*, 443 (1953); D. B. Sprinson and E. Chargaff, J. Biol. Chem., *164*, 433 (1946).

[9]H. R. V. Arnstein, M. Clubb and P. T. Grant, *Radioisotope Conference, 1954*, Vol. I, Academic Press, New York, 1954, p. 306.

VALINE-1-C^{14}
(2-Amino-3-methylbutyric-1-C^{14} Acid)

$$(CH_3)_2CHCHO \xrightarrow[NH_4Cl]{KC^*N} (CH_3)_2CHCHC^*N \xrightarrow{HCl} (CH_3)_2CHCHC^*OOH$$

NH_2 (a) NH_2 (b)

R. E. Selff and B. M. Tolbert, Atomic Energy Commission Report, UCRL-1301; Nuc. Sci. Abstracts, *5*, 5153 (1951).

A. Procedure (Note 1)

(a) *2-Amino-3-methylbutyronitrile-1-C^{14}*. Approximately 0.8 mmole of hydrogen cyanide-C^{14} (Note 2) is vacuum-distilled into a 100-ml. round-bottomed flask containing 1.28 g. of potassium cyanide (97%), 1.8 ml. of freshly distilled isobutyraldehyde, 0.8 ml. of *N* potassium hydroxide, 1.3 g. of ammonium chloride and 40 ml. of water. The flask is closed, removed from the manifold and placed in an ice-bath for 20 minutes, with occasional shaking. The mixture is heated under reflux at 70–80° for 24 hours, then cooled and extracted with four 25-ml. portions of methylene chloride, and the solvent is evaporated on a steam-bath (Note 3).

(b) *Valine-1-C^{14}*. The residual crude nitrile is refluxed with 50 ml. of concentrated hydrochloric acid for 24 hours on a steam-bath. The mineral acid is removed under vacuum, the last traces being removed by the evaporation of four 60-ml. portions of water. The crude acid hydrochloride (Note 4) is dissolved in a few ml. of water, and equal portions are placed on two columns (200 × 15 mm.) containing 60 ml. of Dowex-50 cation exchange resin in the acid form. The columns are washed with 300 ml. of water, then eluted with 300 ml. of 2 *N* ammonium hydroxide and washed with 800 ml. of water. The combined eluate and final wash are evaporated to dryness, and the residue is recrystallized from water-alcohol. The mother liquor, after yielding a second crop of valine, is evaporated to dryness, and the residue is vacuum-sublimed to give a third crop. The yield of purified product is 1.196 g., 51.4% based on

potassium cyanide. Analysis by two-dimensional paper chromatography and radioautography indicates that the product is homogeneous (Note 5).

B. Notes

1. The procedure is adapted from the Strecker method for synthesizing α-amino acids described by Lipp;[1] also see Clarke and Bean.[2]

2. The hydrogen cyanide-C^{14} is liberated from the potassium salt by treatment with 6 *N* sulfuric acid.

3. The compound is an unstable yellow oil, very soluble in water, alcohol and ether.

4. Valine hydrochloride melts at 189°.

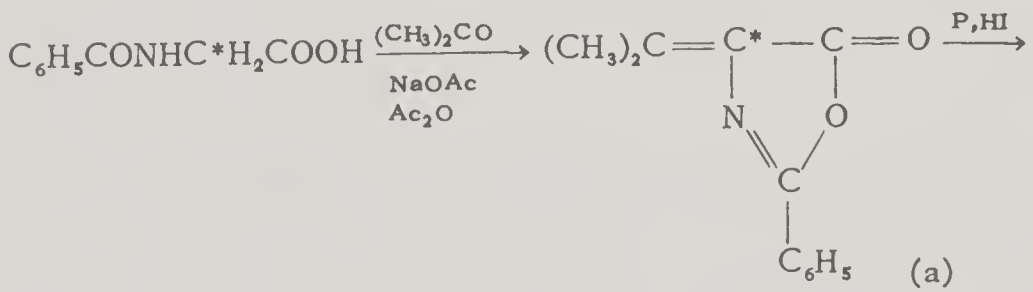
5. The solvents used are butanol-propionic acid-water, and phenol-water. The chromatogram[3] is sprayed with ninhydrin to determine the position of the compound.

[1]Lipp, Ann., *205*, 1 (1880).

[2]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 29.

[3]A. A. Benson, J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas and W. Stepka, J. Am. Chem. Soc., *72*, 1710 (1950).

VALINE-2-C^{14}



P. T. Adams and B. M. Tolbert, J. Am. Chem. Soc., *74*, 6272 (1952).

A. Procedure

(a) *4-Isopropylidene-2-phenyl-2-oxazolin-5-one-4-*C^{14} (Note 1). The hippuric-α-C^{14} acid prepared from 0.76 g. (10.1 mmoles) of glycine-2-C^{14}

is pulverized with 0.6 g. of freshly fused sodium acetate and dissolved in 35 ml. of anhydrous acetone. To the solution is added 5 ml. of acetic anhydride dropwise, and the mixture is refluxed for 15 hours. The hot mixture is poured onto 250 ml. of crushed ice and water, and the product is collected, washed with sodium carbonate solution and dried under vacuum. The yield is 1.16 g., m.p. 98–100°, 57% based on glycine (Note 2); radiochemical yield 50%.

(b) *Valine-2-*C^{14} (Note 3). To a mixture of 1.16 g. of 4-isopropylidene-2-phenyl-2-oxazolin-5-one-4-C^{14}, 2 g. of red phosphorus and 12.5 ml. of acetic anhydride is added slowly 12.5 ml. of hydriodic acid (d. 1.7). The mixture is refluxed for 20 hours; then the solution is filtered and evaporated to dryness under reduced pressure. The residue is dissolved in 100 ml. of 70% ethanol and passed through a cation-exchange column containing 60 ml. of Dowex-50 (50–100 mesh) in the acid form. The anions are removed by washing the column with 100 ml. of 70% ethanol and 250 ml. of water; then the product is eluted with 250 ml. of 2 *N* ammonium hydroxide and 250 ml. of wash water. The eluate is evaporated to dryness at 100° under vacuum; yield 0.504 g., 4.3 mmoles (74.5%), and radiochemical yield 70.5% (Note 4). Two dimensional paper chromatography and radioautography show the product to be free from isotopic and amino acid contamination.

The method is readily adaptable to the synthesis of valine-1-C^{14}.

B. Notes

1. The procedure is that described by Ramage and Simonsen;[1] also see the synthesis of C_1^{14}-thyroxine. An excellent review of azlactones is given by Carter.[2]

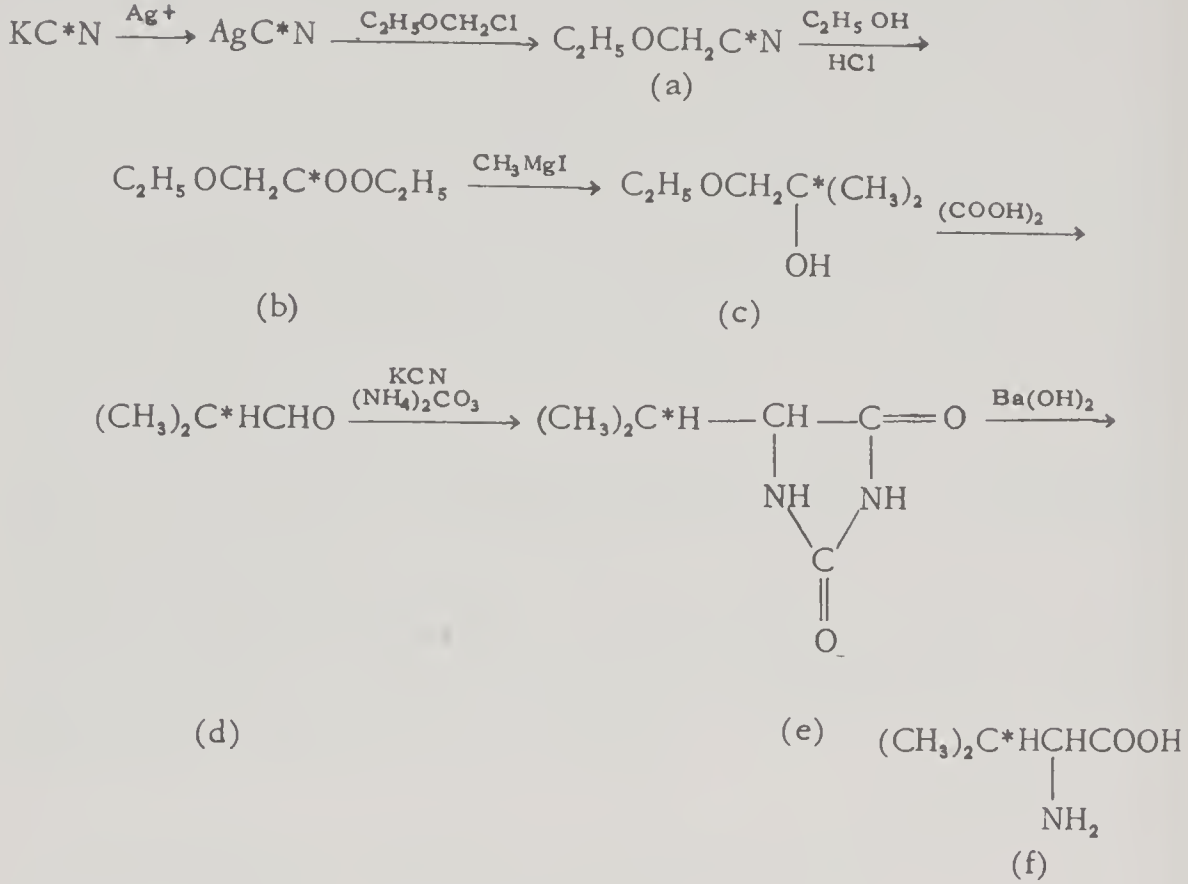
2. From the mother liquor is recovered 0.27 g. of unreacted glycine-2-C^{14} hydrochloride (2.42 mmoles, 23.9%) with a radiochemical recovery of 23%.

3. The procedure is a modification of the method of Gillespie and Snyder.[3]

4. The chemical and radiochemical yields are 56% and 54%, respectively, based on unrecovered glycine.

[1]G. R. Ramage and J. L. Simonsen, J. Chem. Soc., *1935*, 532.
[2]H. E. Carter, *Organic Reactions*, Vol. III, Wiley, New York, 1946, p. 198.
[3]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 489.

VALINE-3-C^{13}



J. Anatol, Compt. rend., *230*, 1471 (1950).

A. Procedure (Note 1)

(a) *Ethoxyacetonitrile-1-C^{13}.* Finely powdered silver cyanide-C^{13} (16.19 g.) is added gradually with vigorous stirring to a 20% excess of chloromethyl ethyl ether. When the reaction is complete, the precipitate is filtered off and washed with ether. The filtrate and ether solution are combined and washed with water until no acid remains (Note 2), then dried over sodium sulfate and distilled; yield 70%, b.p. 134–136°.

(b) *Ethyl Ethoxyacetate-1-C^{13}.* The ethoxyacetonitrile-1-C^{13} is heated for 2 hours on a steam-bath with absolute ethanol saturated with hydrogen chloride. The mixture is cooled and diluted with water, and the product is extracted into ether solution, which is washed, dried and distilled; yield 9.46 g. (Note 3).

(c) *2-Ethoxymethyl-2-propanol-2-C^{13}.* To a stirred ethereal solution of methylmagnesium iodide (2 equiv.) is added dropwise, with cooling, an ethereal solution of ethyl ethoxyacetate-1-C^{13}. The mixture is refluxed for 4 hours, then cooled, and hydrolyzed by the cautious addition of dilute acetic acid. The entire mixture is distilled, and the distillate is saturated with potassium carbonate, then extracted into ether solution,

which is dried over sodium sulfate and fractionally distilled (Note 4). The yield is 6.93 g., 44% based on potassium cyanide.

(d) *2-Methylpropionaldehyde-2-C*^{13}. The 2-ethoxymethyl-2-propanol-2-C^{13} is mixed with 2 molar amounts of anhydrous oxalic acid and heated under reflux at 120–125° for 4–5 hours (Note 5). The product may be isolated by dissolving the excess oxalic acid in water, neutralizing the mixture with sodium carbonate, and then steam-distilling (Note 6).

(e) *5-(1-Methylethyl-2-C*^{13}*)hydantoin* (Note 7). The crude 2-methylpropionaldehyde-2-C^{13}, without isolation, is treated with potassium cyanide and ammonium carbonate according to the procedure of Bucherer[1] or Bergs.[2] The yield is 3.66 g., 48% based on (c) and 21.3% based on potassium cyanide.

(f) *Valine-3-C*^{13} (Note 7). The 5-(1-methylethyl-2-C^{13})hydantoin is hydrolyzed by heating with barium hydroxide solution. The excess barium is removed as barium carbonate by treatment with ammonium carbonate. The filtrate is boiled to decompose the excess ammonium carbonate, then evaporated to dryness; yield 2.29 g., 16.2% based on potassium cyanide (Note 8).

B. Notes

1. The preparation is by the procedure of Sommelet.[3]

2. The chloromethyl ethers are highly reactive compounds and are readily hydrolyzed to formaldehyde, alcohol and hydrochloric acid.

3. The compound boils at 156–157°.

4. The yield of product boiling at 126–130° is 68%. The pure compound boils at 128.5–129° (757 mm.).

5. The decomposition of α-glycol ethers is readily brought about, with loss of alcohol, simply by distilling with dilute mineral acid. The results are particularly good with oxalic acid as dehydrating agent, or with formic acid for high molecular weight compounds.

6. The aldehyde may be extracted from the distillate, dried and fractionally distilled, or worked up as the bisulfite compound. The yield is probably of the order of 90% for this step.

7. Another almost identical synthesis[4] appeared in the literature at the same time.

8. Product may be recovered from the mother liquors as the copper salt.

C. Other Preparations

The preparation of 5-ethyl-5-phenylhydantoin-4-C^{14} by a similar Bucherer synthesis has been described by Vigne;[5] also see 5,5-diphenylhydantoin-4-C^{14}. A mixture of 0.100 g. of ammonium carbonate, 0.100 g. of propiophenone,[6] 0.060 g. of potassium cyanide-C^{14} (25 mg. KC^{14}N + 30

mg. KOH) and 2 ml. of absolute methanol (added last) is sealed in a tube under vacuum (−60°), and heated at 100° for 24 hours. A side arm is then chilled to condense the excess ketone, ammonium carbonate and alcohol, and the tube is sealed off (contents nonisotopic). The product, washed with carbon tetrachloride, is dissolved in 1 ml. of *N* sodium hydroxide and precipitated by the addition of concentrated hydrochloric acid, then centrifuged and dried under vacuum; yield 80%, m.p. 198°. The derivative 5-ethyl-3-methyl-5-phenylhydantoin-4-C^{14} is prepared by direct methylation (90%) by an improvement of the method of Hoffman,[7] by employing methyl sulfate and methanol. The product is separated from unreacted starting material by dissolution in carbon tetrachloride.

[1]H. T. Bucherer and V. A. Libe, J. prakt. Chem., *141*, 5 (1934); Chem. Abstracts, *29*, 127 (1935); E. Ware, Chem. Revs., *46*, 422 (1950); D. T. Mowry, Chem. Revs., *42*, 238 (1948).

[2]H. Bergs, D. R. P. 566,094; Chem. Abstracts, *27*, 1001 (1933).

[3]M. Sommelet, Ann. Chim., (8) *9*, 484 (1906); A. Behal and M. Sommelet, Compt. rend., *138*, 89 (1904).

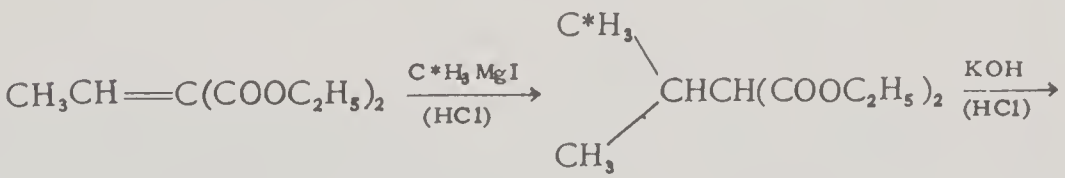
[4]D. P. J. Goldsmith and M. Tishler, U. S. 2,480,644; Chem. Abstracts, *44*, 2017 (1950).

[5]J. P. Vigne and J. Fondarai, Bull. soc. chim. France, (5) *21*, 1221 (1954).

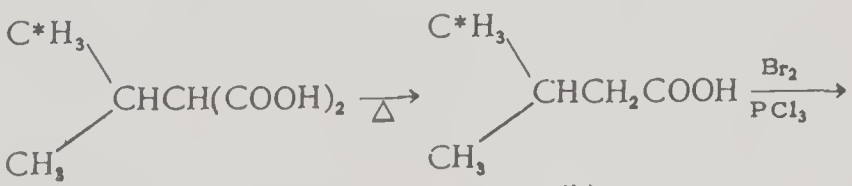
[6]W. T. Read, J. Am. Chem. Soc., *44*, 1746 (1922).

[7]C. Hoffmann, Bull. soc. chim. France, (5) *17*, 45 (1950).

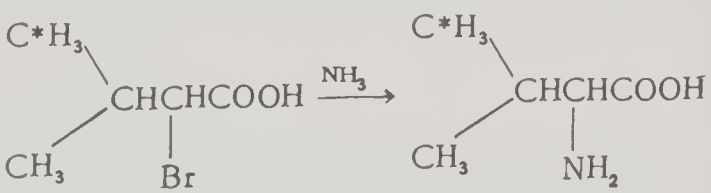
VALINE-4-C^{13}



(a)



(b)



(c) (d)

W. S. Fones, T. P. Waalkes and J. White, Arch. Biochem. Biophys., *32*, 89 (1951).

A. Procedure

(a) *Ethyl (1-Methylethyl-2-C^{13})malonate.* Methylmagnesium-C^{13} iodide is added to ethyl ethylidenemalonate in 88% yield (Note 1).

(b) *3-Methylbutyric-4-C^{13} Acid.* The ester is saponified, and the (1-methylethyl-2-C^{13})malonic acid is decarboxylated, after the method of Marvel,[1] giving the product in 86% yield.

(c) *2-Bromo-3-methylbutyric-4-C^{13} Acid* (Note 3). 3-Methylbutyric-4-C^{13} acid is brominated (92% yield) with bromine in the presence of phosphorus trichloride according to the method of Marvel.[2]

(d) *Valine-4-C^{13}.* 2-Bromo-3-methylbutyric-4-C^{13} acid is aminated in liquid ammonia, in 80–85% yield, after the procedure of Sisler and Cheronis,[3] which follows (Note 4).

The α-bromoisovaleric acid is sealed in a tube (cooled in a Dry Ice-bath) with 20 molar amounts of liquid ammonia; then the mixture is stored at room temperature for about 24 hours. The ammonia is evaporated, and the residue is crystallized from water by the addition of alcohol (Note 5).

B. Notes

1. Directions are given by Fones for preparing the starting material (78% yield) from ethyl malonate, paraldehyde and acetic anhydride.

No directions are given for the synthesis, but in an analogous reaction,[4] a solution of 216 g. of ethyl ethoxymethylenemalonate in 150 ml. of ether is added over a period of 2 hours to a cooled, stirred Grignard solution prepared from 272 g. of ethyl bromide in 300 ml. of absolute ether and 61 g. of magnesium in 400 ml. of ether. The cooled mixture is is poured slowly into 215 ml. of 12 *N* hydrochloric acid and 1 kg. of ice. The aqueous layer is separated and extracted with three 200-ml. portions of ether. The combined extract is dried over magnesium sulfate and concentrated, and the residue is distilled at atmospheric pressure.

2. Experiment shows that better yields are obtained when bromination follows the decarboxylation, rather than the inverse.[1]

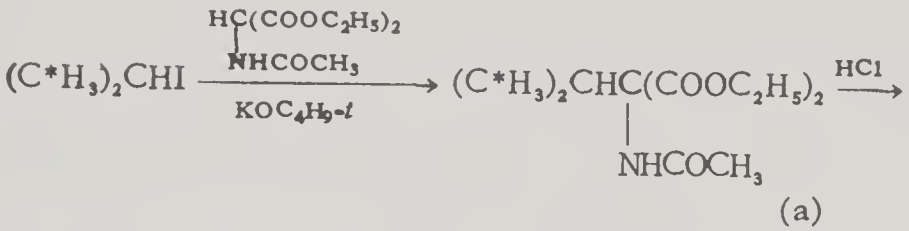
3. Amination in aqueous ammonia gives a yield of only 50%. Sisler and Cheronis[3] have studied the reaction rates and the effects of mole ratio, halogen, ammonium salts and length of carbon chain on the course of ammonolysis of various α-halogen acids in liquid ammonia.

4. L-Valine-4-C^{13} has been prepared in 81% yield by the asymmetric enzymatic hydrolysis of *N*-chloroacetyl-DL-valine-4-C^{13} by the method of Price.[5]

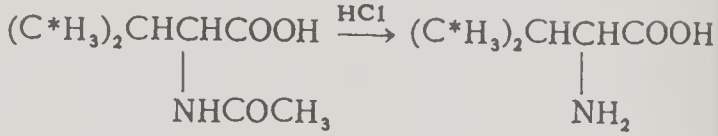
[1] *Organic Syntheses*, Vol. 21, Wiley, New York, 1941, p. 60.
[2] *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 93.
[3] H. H. Sisler and N. D. Cheronis, J. Org. Chem., *6*, 467 (1941).
[4] V. du Vigneaud, G. W. Stacy and D. Todd, J. Biol. Chem., *176*, 907 (1948).
[5] V. E. Price, J. B. Gilbert and J. P. Greenstein, J. Biol. Chem., *179*, 1169 (1949).

2-AMINO-3-METHYL-C^{14}-BUTYRIC-4-C^{14} ACID

(Valine-4,4′-C_2^{14} Hydrochloride)



(a)



(b)

R. Ostwald, Atomic Energy Commission Report, UCRL-2154; Nuc. Sci. Abstracts, 7, 3404 (1953).

A. Procedure (Note 1)

(a) *Acetamidо(1-methyl-C^{14}-ethyl-2-C^{14})malonate.* To a stirred mixture of 26 mmoles of ethyl acetamidomalonate and 20 ml. of dried *t*-butyl alcohol containing 13.2 mmoles of potassium *t*-butoxide is added slowly 13.2 mmoles of 2-iodopropane-1,3-C_2^{14}. The mixture is refluxed for 24 hours; then the salt is removed by centrifugation, and the solvent is distilled off under vacuum.

(b) *Valine-4,4′-C_2^{14} Hydrochloride.* The condensation product is hydrolyzed and decarboxylated by refluxing with concentrated hydrochloric acid for 72 hours. The solution is distilled to dryness at reduced pressure, and traces of acid are removed by distilling three small portions of added water. The crude product (Note 2) is applied to a Dowex-50 ion exchange column (Note 3), which is then washed with 1500 ml. of water (Note 4) and eluted with *N* hydrochloric acid at a flow rate of 20 ml. per hour (Note 5). The fractions containing pure product are combined, evaporated on a steam-bath and dried under vacuum; yield 1.07 g., 54% based on the halide. The molar specific activity is in agreement with that of the starting material. The radiochemical yield is 58%. Two-dimensional paper chromatography and radioautography show the product to be pure (Note 6).

B. Notes

1. The procedure is a modification of the general method of α-amino acid synthesis described by Snyder, Shekleton and Lewis[1] and by Al-

bertson and Archer;[2] see leucine-3-C^{14} and 3-phenyl-1,3,5-C_3^{14}-alanine. Attempts to alkylate ethyl acetamidomalonate by the original procedure[1] were unsuccessful.

2. The crude valine (73% of the initial activity) contains nonisotopic glycine, small amounts of C^{14}-alanine, "C^{14}-2-aminobutyric acid" and less than 3% of a ninhydrin negative unknown C^{14}-compound.

3. The 2.5 × 160-cm. column contains 700 cc. of resin (100–200 mesh) in the hydrogen form, with a capacity of 2100 meq. Due to swelling of the resin, the use of industrial glass pipe is recommended.

4. The effluent possesses practically no activity.

5. Periodic fractions are assayed for activity and identified by chromatography on paper with butanol-propionic acid-water solvent. The contaminants are contained in the first 3190 ml. of eluate, while the product is collected in the following 1555 ml.

6. An additional crop of less pure product is recovered from the previous 320 ml. of eluate in 15% radiochemical yield. A pure sample (6.0 μc./mg.) stored in the dark at room temperature for 15 months has been found[3] to have undergone 1–2% decomposition as a result of self-irradiation. A degradation is described by Wang[4] and Gros.[5]

C. Other Preparations

Norvaline-3-C^{14} hydrochloride is prepared from propyl-1-C^{14} iodide by a similar procedure; yield 74%, radiochemical yield 75% at the 16.6-mmole scale. The product contains 2% of isotopic impurities: alanine (0.4%), 2-aminobutyric acid (0.4%), and an unknown ninhydrin-negative contaminant which appears to originate in the ion exchange process. The spontaneous decomposition[3] of the dry hydrochloride (17.7 μc./mg.) is 5% in 25 months.

Norleucine-3-C^{14} hydrochloride is prepared similarly from butyl-1-C^{14} bromide; yield 62%, radiochemical yield 69%. The product contains 2% of the unknown isotopic, ninhydrin-negative contaminant. The spontaneous decomposition[3] of the dry hydrochloride (17.4 μc./mg.) is 2% in 23 months.

[1]H. R. Snyder, J. K. Shekleton and C. D. Lewis, J. Am. Chem. Soc., 67, 310 (1945).

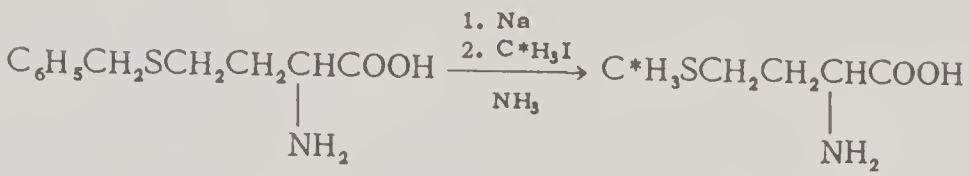
[2]N. F. Albertson and S. Archer, *ibid.*, 67, 308 (1945); N. F. Albertson, *ibid.*, 68, 450 (1946).

[3]B. M. Tolbert, P. T. Adams, E. L. Bennett, A. M. Hughes, M. R. Kirk, R. M. Lemmon, R. M. Noller, R. Ostwald and M. Calvin, J. Am. Chem. Soc., *75*, 1867 (1953); R. M. Lemmon, Nucleonics, *11* (10), 44 (1953).

[4]C. H. Wang, B. E. Christensen and V. H. Cheldelin, J. Biol. Chem., *213*, 365 (1955).

[5]P. Gros, G. P. Talwar and J. Coursaget, Bull. Soc. Chim. Biol., *36*, 1569 (1954).

L-2-AMINO-4-(METHYLTHIO-C^{14})BUTYRIC ACID (C_1^{14}-L-METHIONINE)



D. B. Melville, J. R. Rachele and E. B. Keller, J. Biol. Chem., *169*, 419 (1947).

A. Procedure

To a solution of 1.55 g. of anhydrous *S*-benzyl-L-homocysteine[1] in 30 ml. of dry liquid ammonia, cooled with a Dry Ice-bath and stirred by the slow bubbling of dry nitrogen through the mixture (Note 1), is added sodium wire in small portions until a slight blue color persists for several minutes. The solution is cooled with a bath at $-50°$, and 0.37 ml. of methyl-C^{14} iodide is swept into the reaction mixture with the stream of nitrogen. Small increments (0.025 ml.) of nonisotopic methyl iodide then are added to react with the excess homocysteine, until the nitroprusside test on small aliquots of the mixture becomes negative for the thiol group (Note 2). The solvent is evaporated slowly with the stream of nitrogen passing through the system. The residue is taken up in 15–20 ml. of water and treated with hydriodic acid until the pH is acid to litmus and alkaline to Congo red. The filtered solution is concentrated under vacuum to 5–10 ml., then heated to solution, diluted with 100 ml. of boiling absolute ethanol and stored overnight at 5°. The product is collected, washed with alcohol and ether, and dried. The yield of analytically (Note 3) and optically pure product is 0.860 g., m.p. 283° (dec.) (Note 4).

B. Notes

1. A photograph of the apparatus is presented by Melville. The method is that of Patterson and du Vigneaud;[2] also see Tarver and Schmidt,[3] and 2-amino-4-(ethylthio-1-C^{14})butyric acid.

2. Difficulty is experienced in obtaining a pure product unless an excess of methyl iodide is employed.

3. A specific colorimetric determination for methionine is described by McCarthy.[4]

4. $[\alpha]_D^{22}$-7.75° (2 dm. tube, *c* 0.95, in water). The radiochemical yield is 51% based on barium carbonate. Wang[5] has reported an isotopic preparation from 0.42 ml. of methyl-C^{14} iodide in 83% yield (0.855 g., m.p. 283° dec.) based on the *S*-benzyl-L-homocysteine used.

A degradation of C^{14}-methionine by hydrolysis with hydriodic acid is described by Arnstein.[6]

A method of isolating methionine as methionine methylsulfonium bromide has been described for tracer work by Weiss.[7] The formation of the derivative 2-methyl-C^{14}-2-thiopseudourea picrate, m.p. 221°, is described by Levine, see C_1^{14}-ethionine, Note 5.

The paper chromatography of methionine may be carried out with the same systems described for *S*-ethyl-1-C^{14}-L-homocysteine (q.v.); R_f 0.57 and 0.53, respectively.

C. Other Preparations

From methyl-C^{14}-H_3^2 iodide has been prepared[8] L-2-amino-4-(methylthio-C^{14}-H_3^2)butyric acid.

Challenger[9] has reported the preparation of DL-2-amino-4-(methylthio-C^{14})butyric acid in 48% isotopic yield by this method. Paper chromatography with 80% phenol, or butanol-acetic acid-water, gave two spots with ninhydrin, due to methionine and its sulfoxide, which is formed by oxidation during chromatography. Both the D- and L-forms are prepared from the appropriate optically active starting material; $[\alpha]_D^{20}$ +7.75° and −7.6° in 1% aqueous solution. The compounds were assayed as the picrates.

In a similar manner from *S*-benzyl-D-homocysteine[1] is prepared[10] D-2-amino-4-(methylthio-C^{14})butyric acid, $[\alpha]_D^{25}$ -23.5° as a 1% solution in *N* hydrochloric acid.

The preparation of L-2-amino-4-(methylthio-C^{14})butyric acid by the reduction of L-homocystine with sodium in liquid ammonia followed by coupling with methyl-C^{14} iodide has been reported by Canellakis.[10] The starting material is prepared from L-methionine.

[1]V. du Vigneaud and W. I. Patterson, J. Biol. Chem., *109*, 97 (1935); W. I. Patterson and V. du Vigneaud, *ibid.*, *111*, 393 (1935); L. W. Butz and V. du Vigneaud, *ibid.*, *99*, 135 (1932).

[2]W. I. Patterson and V. du Vigneaud, J. Biol. Chem., *123*, 327 (1938).

[3]H. Tarver and C. L. A. Schmidt, *ibid.*, *130*, 67 (1939).

[4]T. E. McCarthy and M. X. Sullivan, *ibid.*, *141*, 871 (1941).

[5]C. H. Wang, T. E. King, V. H. Cheldelin and B. E. Christensen, *ibid.*, *188*, 753 (1951).

[6]H. R. V. Arnstein and A. Neuberger, Biochem. J., *55*, 259 (1953).

[7]S. Weiss, E. I. Anderson, P. T. Hsu and J. A. Stekol, J. Biol. Chem., *214*, 239 (1955).

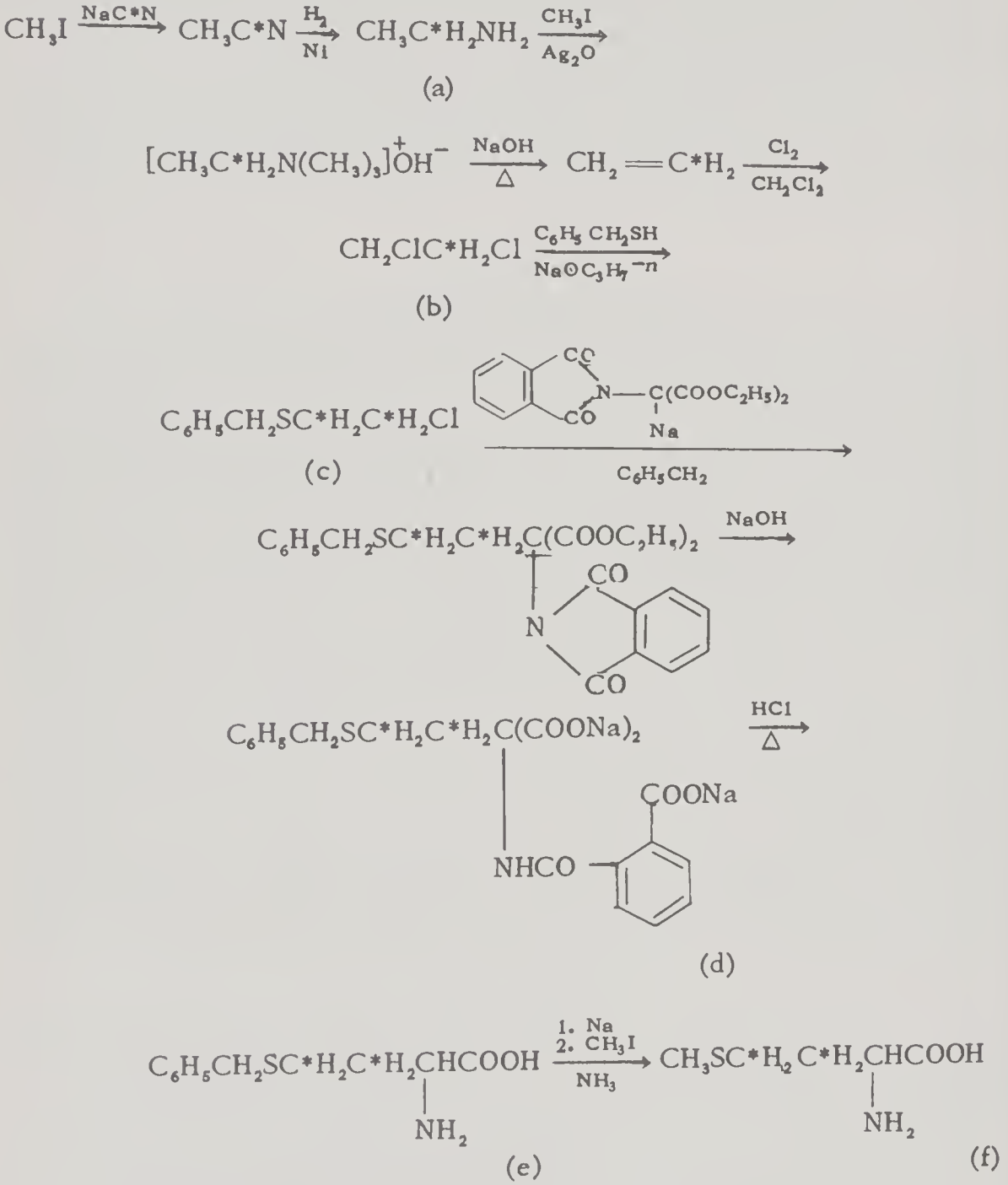
[8]J. R. Rachele, E. J. Kuchinskas, F. H. Kratzer and V. du Vigneaud, J. Biol. Chem., *215*, 593 (1955).

[9]F. Challenger, D. B. Lisle and P. B. Dransfield, J. Chem. Soc., *1954*, 1760.

[10]E. S. Canellakis and H. Tarver, Arch. Biochem., *42*, 387 (1953).

METHIONINE-3,4-$C^{13}_{1/2}$

[2-Amino-4-(methylthio)butyric-4,4-$C^{13}_{1/2}$ Acid]



G. W. Kilmer and V. du Vigneaud, J. Biol. Chem., *154*, 247 (1944).

A. Procedure (Note 1)

(a) Ethylamine-1-C^{13} (Note 2). A mixture of 11.3 g. of 96% sodium cyanide-C^{13}, 17 ml. of water and 36.3 g. of methyl iodide is shaken for 24 hours, then stored overnight in a refrigerator. Sufficient water is

added to dissolve the small amount of precipitate, and the acetonitrile-1-C^{13} is distilled slowly, the fraction boiling at 76–100° (13.8 g.) being collected, diluted to 75 ml. and hydrogenated over 10 g. of Raney nickel (Note 3). The mixture is cooled in an ice-bath, and the catalyst is filtered off and washed with a total of ten portions of water and 5 *N* sodium hydroxide; yield approximately 70% (Note 4).

(b) *1,2-Dichloroethane-C_1^{13}, (Ethylene-C_1^{13} Chloride)* (Note 5). To an ice-cooled, 10% aqueous solution of 11 g. of ethylamine-1-C^{13}, contained in a three-necked flask equipped with a sealed stirrer, reflux condenser and dropping funnel, is added 23.6 g. of sodium hydroxide in small portions. The bath is removed, and 65 ml. of methyl iodide is added in 4-ml. portions, as fast as it is consumed under gentle refluxing (Note 6). The mixture is stirred for 1 hour at room temperature and for 2 hours under reflux. The excess methyl iodide is distilled off, and silver oxide, freshly prepared from 220 g. of silver nitrate, is added slowly with stirring and cooling. The silver iodide is filtered off and washed by suspension in four portions of water. The combined solutions are concentrated to 200 ml. at 70° and 15 mm. The solution of ethyl-1-C^{13}-trimethylammonium hydroxide is filtered into a 250-ml. distilling flask, which is sealed in series to: a condenser, an ice-cooled receiver (arranged so that evolved gas bubbles through 6 *N* sulfuric acid), a bubble trap cooled in a Dry Ice-bath (to condense moisture) and three similarly-cooled bubble traps, each containing 7 ml. of methylene chloride and 7 ml. of dry chlorine (to absorb ethylene-C_1^{13}). The outlet is connected to a calcium chloride drying tube and a bubble counter. The distillation flask is equipped with a nitrogen inlet and a mercury U-tube pressure indicator. The solution is heated at 130–140° while the water distills into the acid. The bath temperature is raised to 140–150° for 4 hours, then gradually to 210° and finally to 250° for a few minutes (Note 7). The excess chlorine is evaporated at room temperature, and the residues are combined, cooled in an ice-bath and decolorized by shaking with one-half saturated sodium bisulfite solution. The organic layer is separated, dried over magnesium sulfate and slowly distilled; the intermediate fractions being redistilled (Note 8). The yield is 19.3 g. (80%), b.p. 77–86°.

(c) *Benzyl 2-Chloroethyl-$C_{1/2}^{13}$ Sulfide* (Note 9). The apparatus consists of a 15-ml. reaction cup equipped with a stirrer, a dropping funnel protected by a calcium chloride tube, a drain (controlled by a pinch-clamp) to a 125-ml. Claisen flask and an inlet tube fed by the liquid refluxing from a cold finger condenser in the side arm of the flask, Figure II, 8. A solution of 2.2 g. of sodium and 11.8 g. of α-toluenethiol in 50 ml. of anhydrous propyl alcohol is added slowly over a period of about

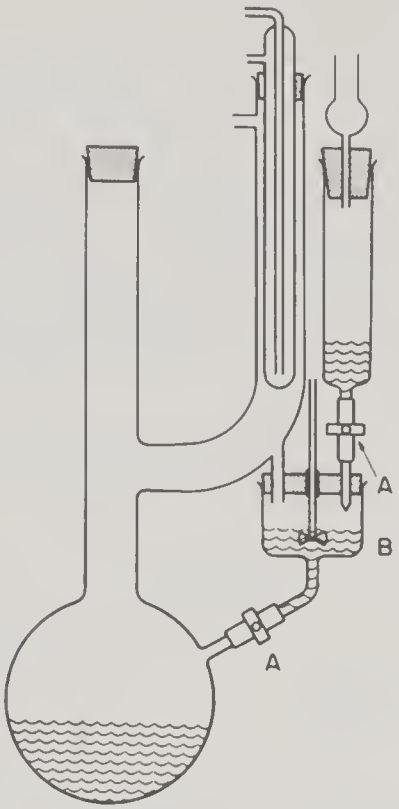


Fig. II, 8. Apparatus for maintaining a local excess of a labeled reagent during the preparation of benzyl 2-chloroethyl sulfide (G. W. Kilmer and V. du Vigneaud). A, drain controller by pinch-clamp; B, reaction cup.

10 hours to 8.9 g. of ethylene-C_1^{13} chloride contained in the flask which is heated at 100–150°. The less volatile product collects in the flask, while the accumulated solvent and unchanged ethylene chloride reflux from the condenser and drop into the cup to react with fresh mercaptide solution. The reaction mixture is poured into 500 ml. of water, and the aqueous phase is separated and extracted with four 25 to 50-ml. portions of benzene. The combined organic layers are concentrated under vacuum, and the residue is distilled; yield 9.8 g. (58%), b.p. 105–108° (1.5 mm.) (Note 10).

(d) *Sodium* [2-(*Benzylthio*)*ethyl*-$C_{1/2}^{14}$](o-*carboxybenzamido*)*malonate.* A mixture of 8.3 g. of benzyl 2-chloroethyl-$C_{1/2}^{13}$ sulfide, 15.7 g. of freshly prepared ethyl sodiumphthalimidomalonate and 1–2 ml. of dry toluene, in a 100-ml. centrifuge tube closed with a calcium chloride tube, is heated for 5 hours with an oil-bath at 170°. The mixture is centrifuged, the oily ethyl α-[2-(benzylthio)ethyl-$C_{1/2}^{13}$]-1,3-dioxo-2-isoindolinemalonate is separated, and the sodium chloride is washed repeatedly with toluene (Note 11). The solvent is evaporated under vacuum from the combined solutions, and the residue is heated for 2 hours at 100° with 19.5 ml. of alcohol and 50.5 ml. of 5 *N* sodium hydroxide (Note 12). The mixture is cooled in an ice-bath, and the solid is filtered off and washed with two

portions of 15:1 alcohol-water. The filtrate is evaporated, and the solid is collected and washed as above. The total yield of crude product is 21.4 g. (Note 13).

(e) *S-Benzylhomocysteine-3,4-$C^{13}_{1/2}$, [2-Amino-4-(benzylthio)butyric-3,4-$C^{13}_{1/2}$ Acid]*. A stirred mixture of the crude salt, 222 ml. of water and 26.3 ml. of concentrated hydrochloric acid is heated for 2 hours on a steam-bath. Concentrated hydrochloric acid (135 ml.) is added and heating is continued for 45 minutes. The mixture is cooled, treated with 1 g. each of infusorial earth and decolorizing carbon and filtered (Note 14). The filtrate is concentrated to 125 ml. under reduced pressure, then cooled in an ice-bath and refiltered. The cooled filtrate is adjusted with ammonia to a pH acid to litmus and alkaline to Congo red, then stored in a refrigerator. The product is collected, washed with ice-water, alcohol and ether; yield 7.0 g., 70% based on benzyl 2-chloroethyl sulfide.

(f) *Methionine-3,4-$C^{13}_{1/2}$*. *S*-Benzylhomocysteine-3,4-$C^{13}_{1/2}$ is converted to methionine by the method of Patterson and du Vigneaud,[1] see L-2-amino-4-(methylthio-C^{14})-butyric acid.

B. Notes

1. The procedure is an adaptation of the methods of Tarver and Schmidt[2] and of Patterson and du Vigneaud.[1]

2. The procedure is adapted from the method of Auger[3] and has been used by Roberts[4] to prepare ethylamine-1-C^{14} in 68% yield. An alternative preparation of acetonitrile-1-C^{14} from methyl sulfate, in 90% yield, has been described by Brown (see acetonitrile-2-C^{14}, Other Preparations).

3. Approximately the theoretical amount of hydrogen is absorbed during 4 hours of shaking.

4. The derivative *N*-ethyl-*p*-bromobenzenesulfonamide is prepared from a small aliquot for assay.

5. The procedure is adapted from the methods of Lossen[5] and Bahr[6].

6. Additional sodium hydroxide may be required to maintain alkalinity toward the end.

7. Most of the ethylene-C^{13}_1 is evolved at 140–150°. Back titration of the acid indicates that nearly the theoretical amount of base has reacted.

8. Methylene chloride, b.p. 41°; ethylene chloride, b.p. 84°.

9. The procedure is a modification of the method of Tarver and Schmidt.[2] The need for 5–10 moles excess of isotopic ethylene chloride is eliminated by the development of a device (Figure II,8) for continuous circulation of the compound. The product is a strong skin vesicant.

10. The product may be prepared in 56% yield by pouring the mercaptide in absolute ethanol into ice-cold 1-bromo-2-chloroethane and stirring for 5 minutes.

11. The ester has not been crystallized.

12. The lumps which form during hydrolysis are crushed at intervals.

13. The free acid,[2] crystallized from cold water by acidification with cold hydrochloric acid, melts at 110–111° (cor.) (dec.).

14. Phthalic acid and a small amount of oil are removed.

[1]W. I. Patterson and V. du Vigneaud, J. Biol. Chem., *123*, 327 (1938).
[2]H. Tarver and C. L. A. Schmidt, J. Biol. Chem., *130*, 67 (1939).
[3]V. Auger, Compt. rend., *145*, 1287 (1907).
[4]J. D. Roberts and J. A. Yancey, J. Am. Chem. Soc., *74*, 5943 (1952).
[5]W. Lossen, Ann., *181*, 364 (1876).
[6]H. Bahr and H. Zieler, Angew. Chem., *43*, 233 (1930).

2-AMINO-4-(ETHYLTHIO-1-C^{14})BUTYRIC ACID
(*S*-Ethyl-1-C^{14}-homocysteine)
(C_1^{14}-Ethionine)

$$(\text{-SCH}_2\text{CH}_2\underset{\displaystyle \text{NH}_2}{\underset{|}{\text{CH}}}\text{COOH})_2 \xrightarrow[\text{Na, NH}_3]{\text{CH}_3\text{C*H}_2\text{I}} \text{CH}_3\text{C*H}_2\text{SCH}_2\text{CH}_2\underset{\displaystyle \text{NH}_2}{\underset{|}{\text{CH}}}\text{COOH}$$

M. Levine and H. Tarver, J. Biol. Chem., *192*, 835 (1951).

A. Procedure (Note 1)

To a solution of 2.73 mmoles of homocystine (Note 2) in 40 ml. of liquid ammonia, stirred by a gentle stream of gaseous ammonia is added sodium in small portions until the color persists for 2–4 minutes. Ethyl-1-C^{14} iodide (5.2 mmoles) is added, and ammonia is bubbled through for a few minutes. The solvent is evaporated, the last traces being removed under reduced pressure; then the residue is dissolved in water and neutralized with hydriodic acid. The product is precipitated with alcohol, collected, washed with alcohol-ethyl iodide and ether, then dried; crude yield 78% based on ethyl iodide used (Note 3). Purification is achieved by dissolution in hot water, treatment with carbon and two reprecipitations with alcohol (Note 4).

B. Notes

1. The procedure is a modification of the method of Dyer.[1] *S*-Ethyl-1-C^{14}-homocysteine has been prepared in a similar manner by Stekol and Weiss,[2] by ethylation of the sodium salt of homocysteine in liquid ammonia. The salt may conveniently be prepared by reducing *S*-benzylhomocysteine with sodium in liquid ammonia; see L-2-amino-4-(methylthio-C^{14}) butyric acid.

2. Several preparations of homocystine are described by Patterson.[3]

A diagram of a convenient vacuum-jacketed reaction vessel, with suitable gas facilities for conducting preparations in liquid ammonia without a cooling bath, is presented by Levine.

3. The chief contaminant is nonisotopic starting material. Homocystine melts at 260–265°(dec.); the dibenzoyl derivative melts at 184–185° (dec.).

4. Ethionine, heated[1] at the rate of 20° per minute, begins to melt at 272° and decomposes at 284° (cor.); the *N*-benzenesulfonyl derivative melts at 80°(cor.).

A degradation is described by Levine and Tarver. The compound is dealkylated in constant-boiling hydriodic acid[4] and the dried ethyl-1-C^{14} iodide is reacted with the calculated amount of thiourea in absolute alcohol followed by an equivalent amount of picric acid, giving 2-ethyl-1-C^{14}-2-thiopseudourea picrate, m.p. 186°. The solubility is 0.00793 g. / ml. of 95% alcohol at 4°.

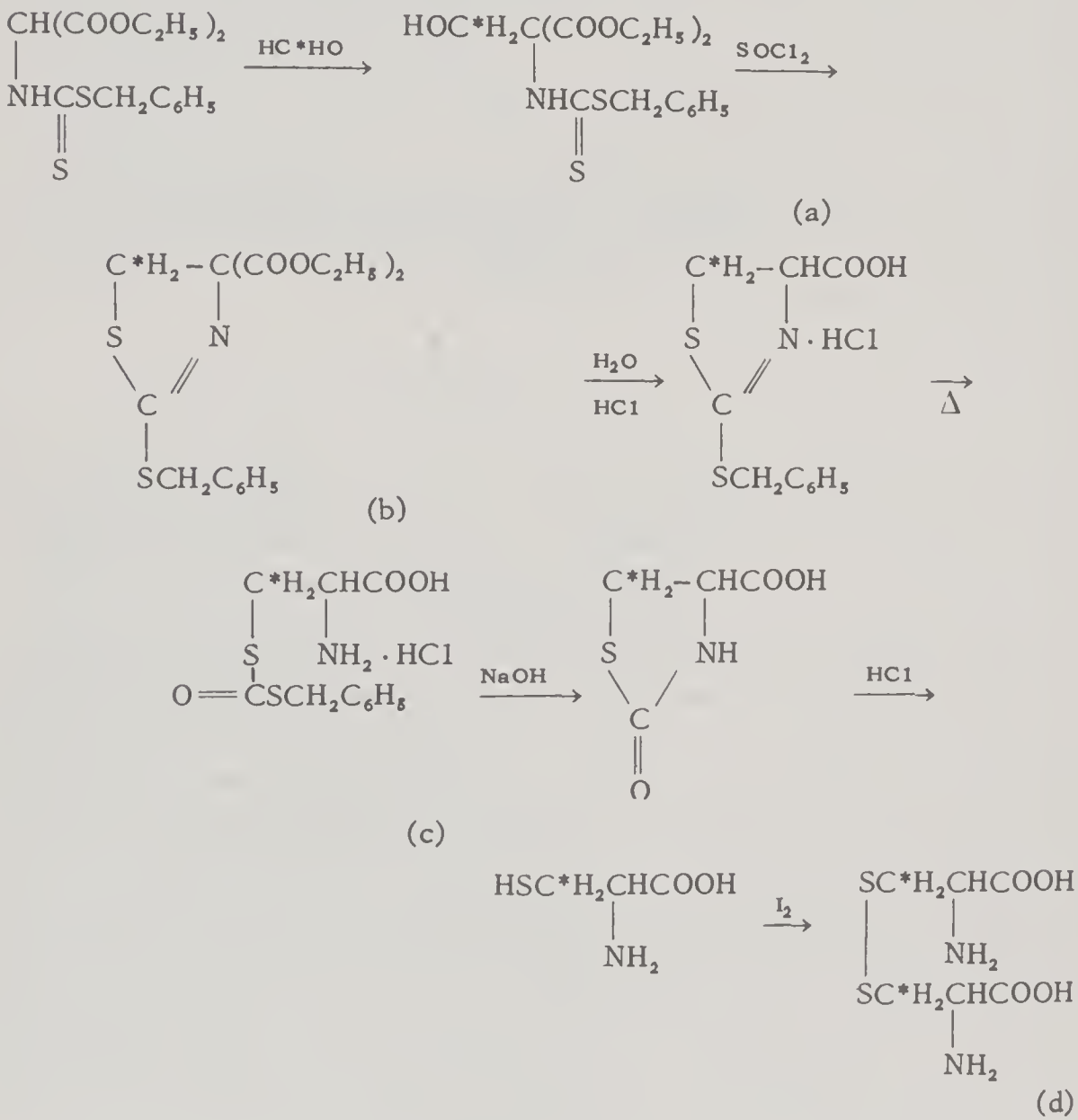
C. Other Preparations

The preparation of *S*-ethyl-1-C^{14}-L-homocysteine from ethyl-1-C^{14} iodide and *S*-benzyl-L-homocysteine has been described by Gross.[5] The starting material (1.14 g.), prepared from methionine through *S*-benzyl-DL-homocysteine[6] and *N*-acetyl-*S*-benzyl-DL-homocysteine,[7] was reduced with sodium in liquid ammonia, and the mercaptide was reacted with 0.63 g. of ethyl-1-C^{14} iodide. The apparatus is a modification of that referred to in Note 3. The residue left by the evaporation of ammonia was dissolved in water, and the solution was adjusted to pH 1–2 with hydrochloric acid and placed on a column[8] of Dowex 50 (2.5 × 22 cm., 200–400 mesh, 8% cross-linked). The fractions containing product eluted with 2.5 *N* hydrochloric acid were evaporated under reduced pressure, and the residue was dissolved in water and adjusted to pH 6.2 with sodium hydroxide. Purification was accomplished by sublimation at 180–200° (1 mm.); yield 0.400 g. (60%), $[\alpha]_D^{25}$ + 23°, 1% concentration in 2 *N* hydrochloric acid. Paper chromatography with 1-butanol-water acetic acid (10:5:2), or *t*-pentyl alcohol-water-pyridine (7:6:8) at 22–25° on Whatman No. 4 paper indicated that the product was free of amino acid and radiochemical impurities; R_f 0.67 and 0.61, respectively.

[1]H. M. Dyer, J. Biol. Chem., *124*, 519 (1938).
[2]J. A. Stekol and K. Weiss, *ibid.*, *185*, 577 (1950).
[3]W. I. Patterson and V. du Vigneaud, *ibid.*, *111*, 393 (1935).
[4]H. D. Baernstein, J. Biol. Chem., *115*, 25 (1936).
[5]D. Gross and H. Tarver, J. Biol. Chem., *217*, 169 (1955).
[6]C. A. Dekker and J. S. Fruton, J. Biol. Chem., *173*, 471 (1948).
[7]S. M. Birnbaum and J. P. Greenstein, Arch. Biochem. Biophys., *42*, 212 (1953).

[8]W. H. Stein and S. Moore, *Cold Spring Harbor Symposia Quant. Biol.*, *14*, 179 (1950); O. G. Lien, Jr., E. A. Peterson and D. M. Greenberg, Anal. Chem., *24*, 920 (1952).

CYSTINE-3,3′-C_2^{14}
[3,3′-Dithiobis(alanine-3-C^{14})]



H. R. V. Arnstein and J. C. Crawhall, Biochem. J., *55*, 280 (1953).

A. Procedure (Note 1)

(a) *Ethyl [(Benzylthio)thiocarbonylamino]hydroxymethyl-C^{14}-malonate.* To a mixture of 1.30 g. (10% excess) of ethyl [(benzylthio)thiocarbon-

ylamino]malonate (Note 2) and 10 ml. of pyridine is added a solution of 0.104 g. of formaldehyde-C^{14} in 5 ml. of water. The mixture is stored overnight at room temperature, then treated with 0.010 g. of formaldehyde in water, stored for 7 hours and finally evaporated to dryness under vacuum below 60°. The residue is dried overnight in a vacuum desiccator over sulfuric acid (Note 3).

(b) *Ethyl 2-(Benzylthio)-2-thiazoline-4,4-dicarboxylate-5-C^{14}.* To a solution of the crude ester in 5 ml. of absolute ether, cooled in an ice-salt bath, is added dropwise 10 ml. of cooled, purified[1] thionyl chloride. The mixture is stored in the cold for 1 hour, then concentrated under vacuum below 20° (Note 4).

(c) *S-[(Benzylthio)carbonyl]cysteine-3-C^{14} Hydrochloride.* The residual ester is made alkaline to lithmus with 5 *N* alcoholic sodium hydroxide. After 20 minutes (Note 5), the mixture is neutralized with 3 *N* hydrochloric acid, and the alcohol is removed under vacuum. The residue is mixed with excess 3 *N* hydrochloric acid (Note 6), and the mixture is refluxed for 2 hours, then evaporated under vacuum to remove the acid (Note 7).

(d) *Cystine-3,3'-C_2^{14}.* The residue is stirred with 50 ml. of aqueous 0.5 *N* sodium hydroxide for 30 minutes (Note 8). The solution is acidified with 50 ml. of concentrated hydrochloric acid and refluxed for 24 hours. The mixture is evaporated to dryness under vacuum, water being added to facilitate the removal of remaining hydrochloric acid and α-toluenethiol. A solution of the residue in 50 ml. of water is washed with two portions of ether and again evaporated to dryness. The residue is extracted thoroughly with alcohol, and the combined filtrate and washings (40 ml.) are diluted with an equal volume of water. The solution is oxidized by titration with 0.2 *N* iodine (10 ml., 52% yield) and starch as an external indicator. Excess iodine is destroyed with sodium thiosulfate solution, and the pH is adjusted to 5 by addition of an excess of concentrated ammonium hydroxide followed by acetic acid. The mixture is stored overnight at 0°, and the product is collected on a filter and dried; yield 0.2193 g., 47.5% based on the malonate (Note 9).

The product is resolved (Note 10) by the isotopic dilution method of Wood;[2] see L-tyrosine-β-C^{14}.

B. Notes

1. The procedure is an adaptation of that described by Crawhall,[3] without isolation and purification of intermediates.

2. Directions are given by Crawhall[3] for preparing the starting material by reaction of carbon disulfide with aminomalonic ester followed by alkylation with benzyl chloride; yield 88%, m.p. 70°.

3. The yield of ester melting at 80° is 86%. Purification by crystallization from aqueous alcohol raises the melting point to 89°.

4. The crude ester may be isolated in 95% yield by ether extraction from sodium carbonate solution.

5. A copious precipitate of sodium 2-(benzylthio)-2-thiazoline-4,4-dicarboxylate-5-C^{14} might be isolated by centrifuging.

6. Carbon dioxide is evolved, and 2-(benzylthio)-2-thiazoline-4-carboxylic-5-C^{14} acid hydrochloride separates, m.p. 165° from glacial acetic acid.

7. The pure hydrochloride melts at 183° (dec.), after recrystallization from 6 *N* hydrochloric acid.

8. Above pH 7, 2-(benzylthio)-2-thiazoline-4-carboxylic-5-C^{14} acid hydrochloride liberates α-toluenethiol and is converted quantitatively to 2-oxo-4-thiazolidinecarboxylic-5-C^{14} acid which may be isolated by acidification, concentration and other extraction; m.p. 155° from ethyl acetate. Compound (c) is rather resistant to acid hydrolysis.

9. The specific activity is unchanged by recrystallization from water, and purity is established by paper chromatography and microbiological assay.

10. The resolution of DL-cystine-3,3′-C_2^{14} through formation of the butanol-insoluble salt of *S*-benzyl-*N*-formyl-D-cysteine-3-C^{14} is described in detail by Arnstein and Grant.[4] The amount of contaminating C_1^{14}-D-diasterioisomer in the mother liquor is reduced to 0.1% by isotopic dilution with carrier nonisotopic D-salt. Hydrolysis, followed by reduction and oxidation, converts the respective salts to the D- and L-cystine-3,3′-C_2^{14} yield 0.281 g. and 0.310 g., respectively, based on 2.71 g. of *S*-benzyl-*N*-formyl-DL-cysteine-3-C^{14}.

The preparation of *N,N*′-bis(phenylacetyl-1-C^{14})-L-cystine, by conversion of phenylacetic-1-C^{14} acid into the acid chloride by reaction with thionyl chloride, followed by acylation[5] of L-cystine, has been reported by Arnstein;[6] yield 76%, m.p. 134–136° from water.

C. Other Preparations

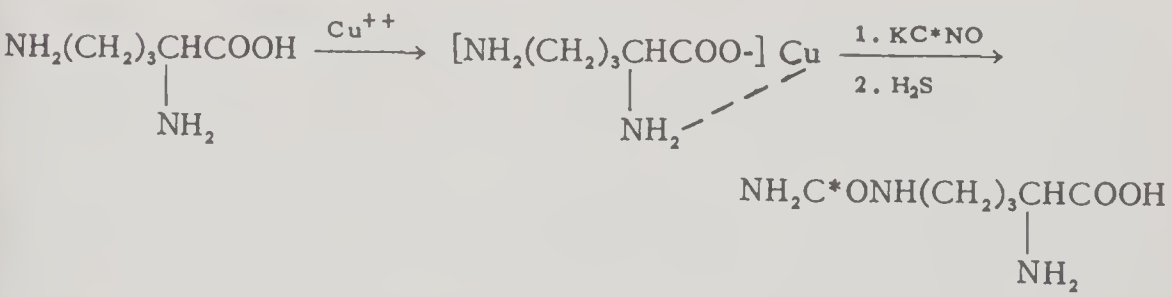
The preparation of 3,3′-dimethylcystine-1,1′-C_2^{14} by a similar method has been outlined by Arnstein.[6] Allothreonine-1-C^{14} was esterified, and the ethyl ester was converted to ethyl 3-hydroxy-2-thiobenzamidobutyrate-1-C^{14} by reaction with [phenyl(thiocarbonylthio)]acetic acid. The ring was closed by the action of thionyl chloride, the ethyl 5-methyl-2-phenyl-2-thiazoline-4-carboxylate-C^{14} was hydrolyzed with hydrochloric acid, and the 3-methylcysteine-1-C^{14} was oxidized to the final *threo*-3,3′-dimethylcystine-1,1′-C_2^{14}.

The preparation of 2,2′-dimethylcystine-1,1′-C_2^{14} has been reported by Arnstein,[6] m.p. 270° (dec.). 2-Amino-3-(benzylthio)-2-methylpropionitrile-

1-C^{14} was prepared from sodium cyanide-C^{14} and (benzylthio)-2-propanone[7] by a Strecker syntheses,[8] and hydrolyzed with concentrated hydrochloric acid. The *S*-benzyl-2-methylcysteine-1-C^{14} was debenzylated with sodium in liquid ammonia (see methionine-3,4-$C^{13}_{1/2}$), and the product was oxidized with air at pH 8 in the presence of ferric ion, and precipitated at pH 5. The purity was checked by paper chromatography, with amyl alcohol-pyridine and 1-butanol-acetic acid as solvents.

[1]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 570.
[2]J. L. Wood and H. R. Gutmann, J. Biol. Chem., *179*, 535 (1949).
[3]J. C. Crawhall and D. F. Elliott, J. Chem. Soc., *1951*, 2071.
[4]H. R. V. Arnstein and P. T. Grant, Biochem. J., *57*, 360 (1954); J. L. Wood and V. du Vigneaud, J. Biol. Chem., *130*, 109 (1939).
[5]L. C. King and F. H. Suydam, J. Am. Chem. Soc., *74*, 5499 (1952).
[6]H. R. V. Arnstein, M. Clubb and P. T. Grant, *Radioisotope Conference, 1954*, Vol. I, Academic Press, New York, 1954, p. 306.
[7]C. Wahl, Ber., *55B*, 1449 (1922).
[8]D. O. Holland and J. H. C. Nayler, J. Chem. Soc., *1952*, 3403.

L-5-UREIDO-C^{14}-NORVALINE
(C^{14}_1-L-Citrulline)
(N^5-Carbamoyl-C^{14}-ornithine)



L. H. Smith, Jr., J. Am. Chem. Soc., 77, 6691 (1955).

A. Procedure

To a test tube containing the crude potassium cyanate-C^{14}, prepared from 2.0 mmoles of urea-C^{14} with 1.2 mmoles of potassium carbonate, is added 2.25 mmoles of copper L(+)-ornithine (Note 1) and 3.5 ml. of water. The mixture is stored at 37° for 5 days (Note 2), and copper C^{14}-citrulline is collected and dissolved in 0.6 *N* hydrochloric acid. The solution is saturated with hydrogen sulfide and filtered to remove cupric sulfide. The chloride ion is removed by passage of the solution through a 1 × 8-cm. column of Dowex-2 (acetate). The eluate is concentrated under reduced pressure and diluted with several volumes of absolute

ethanol. When crystallization commences, a little ether is added, the mixture is stored overnight in a refrigerator, and the product is collected; yield 0.179 g. (51% based on urea), m.p. 218–219° (dec.) (Note 3).

B. Notes

1. L-Ornithine dihydrochloride is adjusted to pH 7 with sodium hydroxide in aqueous solution; then the solution is boiled with an excess of cupric carbonate and filtered. This method of masking the α-amino group of α-amino acids by chelation with cupric ion is that of Kurtz.[1]

2. Copper citrulline begins to precipitate within 2 hours. A time study of yield based on ornithine showed: 24 hrs., 68%; 48 hrs., 74.5%; 72 hrs., 77%; 96 hrs., 78.5%; 120 hrs., 80%. L-Citrulline copper melts[1] at 249–250° (dec.).

3. Yields as high as 60% have been realized. Reaction at 100° for 20 minutes leads to 10–15% lower yields. Paper chromatography[2] with phenol-water (80:20) shows a single ninhydrin spot, R_f 0.62. Recrystallization from aqueous alcohol raises the melting point of citrulline to 230–232° (dec.).

C. Other Preparations

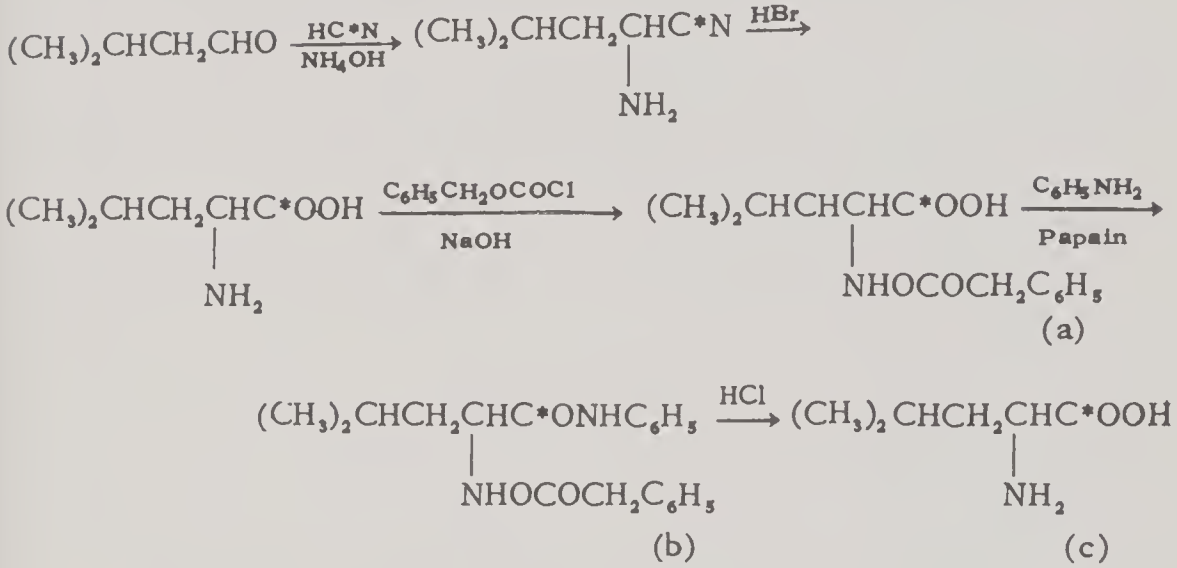
In a similar manner from copper L(+)-lysine is prepared L-5-ureido-C^{14}-norleucine hydrochloride, (C_1^{14}-L-homocitrulline hydrochloride); yield 60% based on urea at the 1-mmole level, m.p. 177–178°. Paper chromatography with phenol-water (80:20) shows a single ninhydrin spot, R_f 0.73.

By the same procedure from L-2,4-diaminobutyric acid copper chelate is prepared L-2-amino-4-ureido-C^{14}-butyric acid; yield 26% based on urea at the 1-mmole scale, m.p. 207–208° (dec.). Paper chromatography with phenol-water (80:20) reveals a single ninhydrin spot, R_f 0.58. L-2,4-Diaminobutyric acid dihydrochloride is prepared from L-glutamic acid by the procedure of Adamson.[3]

The preparation of L-5-ureido-$C^{14}_{1/1}$-5-$N^{15}_{1/1}$-norvaline by condensation of urea-$C^{14}_{1/1}$-$N^{15}_{1/1}$ with L-ornithine copper sulfate in a sealed tube at 100°, by the method of Kurtz,[4] has been reported by Cooper.[5] The precipitated $C^{14}_{1/1}$-$N^{15}_{1/1}$-L-citrulline copper was decomposed with hydrogen sulfide, giving the product (m.p. 220–221°), which gave only one ninhydrin spot with the correct R_f value, following chromatography in phenol-water.

[1] A. C. Kurtz, J. Biol. Chem., *180*, 1253 (1949).
[2] R. J. Block, *Paper Chromatography*, Academic Press, New York, 1952.
[3] D. W. Adamson, J. Chem. Soc., *1939*, 1564.
[4] A. C. Kurtz, J. Biol. Chem., *122*, 477 (1938).
[5] C. Cooper, R. Wu and D. W. Wilson, J. Biol. Chem., *216*, 37 (1955).

L-LEUCINE-1-C^{14}
(L-2-Amino-4-methylvaleric-1-C^{14} Acid)



H. Borsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighley and P. H. Lowy, J. Biol. Chem., *184*, 529 (1950).

A. Procedure (Note 1)

(a) N-*Carbobenzyloxyleucine-1-*C^{14} (Note 2). To the distillate (1 ml.) obtained from a mixture of 0.135 g. of potassium cyanide-C^{14} and 10% sulfuric acid, in a receiver at -20°, is added 0.8 ml. of concentrated ammonium hydroxide. At room temperature then is added 0.530 g. of freshly distilled isovaleraldehyde (Note 3), and the stoppered flask is shaken for 18 hours. The 2-amino-4-methylvaleronitrile-C^{14} is hydrolyzed by refluxing for 30 minutes with 10 ml. of 48% hydrobromic acid, then heating for 4 hours on a steam-bath. The mixture is evaporated in an air stream, and the residue, after drying under vacuum over sulfuric acid and potassium hydroxide, is boiled with 10 ml. of *N* sodium hydroxide until the vapor tests nearly neutral. The solution is filtered, acidified with about 0.1 ml. of 20% hydrochloric acid and concentrated to 2 ml., then made strongly alkaline with 0.3 ml. of 4 *N* sodium hydroxide and cooled in an ice-bath. To the cold solution of crude DL-leucine-1-C^{14} is added 0.6 ml. of benzyl chloroformate (Note 4) and 0.9 ml. of 4 *N* sodium hydroxide in four equal portions (Note 2). The alkaline mixture is washed with ether, then acidified, and extracted with ether, and the extract is dried and evaporated to a syrup; yield 0.410 g.

(b) N-*Carbobenzyloxy-L-leucine-1-*C^{14} *Anilide.* *N*-Carbobenzyloxy-DL-leucine-1-C^{14} (0.410 g.) is resolved through the synthetic antipodal specificity of a proteolytic enzyme according to the general procedure of Berg-

mann and Fraenkel-Conrat,[1] which follows. The yield is 0.253 g. of the L-anilide.

To a solution of the carbobenzyloxy-DL-leucine, prepared from 6.5 g. of DL-leucine, in 44 ml. of *N* sodium hydroxide is added 75 ml. of 2 *N* sodium acetate, 150 ml. of citrate buffer (pH 5), 6.5 ml. of aniline, 1.5 ml. of glacial acetic acid, 0.5 g. of cysteine, 150 ml. of papain solution and water to 500 ml. (Note 5). The mixture is maintained at 40° for 1 week with occasional shaking; then the insoluble product is filtered off in nearly quantitative yield (Note 6).

(c) *L-Leucine-1-*C^{14}. The carbobenzyloxy-L-leucine-*1-*C^{14} anilide (0.253 g.) is refluxed for 3.5 hours with 5 ml. of 6 *N* hydrochloric acid. The mixture is diluted with 15 ml. of water and extracted with ether. The aqueous solution, containing L-leucine-1-C^{14} and aniline hydrochlorides, is evaporated and dried under vacuum over sulfuric acid and potassium hydroxide. The residue, dissolved in 1.75 ml. of water, is mixed with 0.495 g. of carrier L-leucine in 2.75 ml. of *N* hydrochloric acid, and the pH is adjusted to 6 with concentrated ammonium hydroxide. The mixture is diluted with one volume of absolute alcohol and stored for 2 hours at room temperature to crystallize. The product is collected and washed with three portions of absolute alcohol and with ether; yield 0.461 g. (Note 7).

B. Notes

1. The procedure is an adaptation of the Strecker synthesis, and the method of resolution is described by Bergmann and Fraenkel-Conrat.[1]

2. The procedure is adapted from that of Bergmann, Zervas and Fruton.[2]

3. Directions are given by Borsook for preparing isovaleraldehyde from leucine. The addition of hydrogen cyanide to aldehydes is reviewed by Mowry.[3]

4. Directions are given by Carter[4] for preparing and using benzyl chloroformate.

5. Directions are given by Bergmann[1] for preparing the papain activated with cysteine.

6. The mother liquor contains *N*-carbobenzyloxy-D-leucine-1-C^{14}, which may be obtained by ether extraction of the acidified solution. Acid hydrolysis, or catalytic hydrogenation[2] of the hydrochloride in methanol, forms D-leucine-1-C^{14} hydrochloride almost quantitatively. The D-leucine-1-C^{14} may be racemized,[5] by heating with barium hydroxide at 160–170° in a sealed tube, then put through the resolution process again to provide additional amounts of the L-form.

7. Before dilution with carrier, $[\alpha]_D^{26}$ + 15.7°, (c = 3.6 in *N* hydrochloric acid).

C. Other Preparations

The preparation of methionine-2-C^{14} from 3-(methylthio)propionaldehyde-1-C^{14}, cyanide and ammonia has been outlined by Claus.[6]

[1]M. Bergmann and H. Fraenkel-Conrat, J. Biol. Chem., *119*, 707 (1937).
[2]M. Bergmann, L. Zervas and J. S. Fruton, J. Biol. Chem., *115*, 593 (1936).
[3]D. T. Mowry, Chem. Revs., *42*, 231 (1948).
[4]*Organic Syntheses*, Vol. 23, Wiley, New York, 1943, p. 13.
[5]E. Schulze and E. Bossherd, Ber., *18*, 389 (1885).
[6]C. J. Claus and J. L. Morgenthau, Jr., J. Am. Chem. Soc., *73*, 5005 (1951); G. Barger and F. P. Coyne, Biochem. J., *22*, 1417 (1928).

LEUCINE-2-C^{14}

$$(CH_3)_2CHCH_2C^*OONa \xrightarrow{C_{16}H_{33}Br} (CH_3)_2CHCH_2C^*OOC_{16}H_{33} \xrightarrow[CuCr_2O_4]{H_2}$$

$$(CH_3)_2CHCH_2C^*H_2OH \xrightarrow{PBr_3} (CH_3)_2CHCH_2C^*H_2Br \xrightarrow{KCN}$$

(a)

$$(CH_3)_2CHCH_2C^*H_2CN \xrightarrow{NaOH} (CH_3)_2CHCH_2C^*H_2COONa \xrightarrow[2.\ Br_2,\ P]{1.\ HCl}$$

(b)

$$(CH_3)_2CHCH_2\underset{\displaystyle Br}{\underset{|}{C^*}}HCOOH \xrightarrow{NH_3} (CH_3)_2CHCH_2\underset{\displaystyle NH_2}{\underset{|}{C^*}}HCOOH$$

(c) (d)

H. Hauptmann, P. T. Adams and B. M. Tolbert, J. Am. Chem. Soc., *74*, 2423 (1952).

A. Procedure

(a) *1-Bromo-3-methylbutane-1-C^{14}, (Isopentyl-1-C^{14} Bromide).* A mixture of 2.10 g. (16.9 mmoles) of sodium 3-methylbutyrate-1-C^{14} and 5.40 g. (17.7 mmoles) of cetyl bromide is heated in a sealed tube at 280° for 11 hours with shaking. The contents of the tube are dissolved in absolute ether and the solution is filtered (Note 1). The filtrate is evaporated to dryness in a stainless steel reaction vessel (Note 2), leaving crude cetyl 3-methylbutyrate-1-C^{14} in over 95% yield. After addition of 5.5 g. of copper chromite catalyst,[1] the vessel is closed and evacuated to 50μ for 1.5 hours to remove traces of ether. The reactor is filled with hydrogen to a pressure of 170 atmospheres and heated to 250° for 10 hours with shaking. The hydrogen is released through a spiral trap

fitted with a sintered disc and cooled in liquid nitrogen (Note 3). The 3-methyl-1-butanol-1-C^{14} is distilled into the cold trap by warming the reactor 80° and maintaining reduced pressure overnight (Note 4). The carbinol is converted to the bromide with excess phosphorus tribromide[2] (see ethyl-1-C^{14} bromide). The product is washed with water and dried over phosphorus pentoxide; yield 1.88 g. (73%).

(b) *4-Methylvaleric-2-C^{14} Acid, (Isocaproic-2-C^{14} Acid)* (Note 5). The 1-bromo-3-methylbutane-1-C^{14} (1.88 g., 12.4 mmoles) is distilled into a mixture of 1.85 g. (25 mmoles) of potassium cyanide and 0.2 g. of potassium iodide in 25 ml. of ethanol. The mixture is refluxed for 42 hours; then 4 g. of silver sulfate and 30 ml. of water are added, and the 4-methylvaleronitrile-2-C^{14} is slowly steam-distilled. To the distillate is added 15 g. of potassium hydroxide, and the mixture is refluxed for 24 hours. The solution is acidified with 50 ml. of 10 *N* sulfuric acid; the liberated 4-methylvaleric-2-C^{14} acid is collected by steam distillation and titrated with sodium hydroxide solution, and the solution is evaporated. The yield of sodium 4-methylvalerate-2-C^{14} is 0.949 g., 55% (Note 6).

(c) *2-Bromo-4-methylvaleric-2-C^{14} Acid.* Dry hydrogen chloride is passed slowly over 0.508 g. of vacuum-dried sodium 4-methylvalerate-2-C^{14} in a tube attached to a spiral trap cooled to –20° (Note 7). The salt is warmed gently with a burner, and the liberated acid is distilled into the cold trap. The acid is vacuum-distilled at 10μ into a small bromination flask (cooled in liquid nitrogen) by heating to 90–95°. Carrier isocaproic acid (2.7 mmoles) and 0.2 ml. of phosphorus tribromide are added, and the mixture is stored overnight. Red phosphorus (0.04 g.) and and 0.02 g. of iodine are added, and the condenser is filled with Dry Ice and fitted with a Drierite guard-tube. The mixture is heated on a steam-bath for thirty minutes while 2.3 ml. of bromine is added slowly. The reaction mixture is frozen in liquid nitrogen, and the condenser is warmed with hot water to return all material to the reaction flask. Water (5 ml.) is added to the mixture, which then is slowly warmed to room temperature. Another portion of water is added, and the vessel is swept for 4 hours with a gentle air stream to remove bromine. The product is extracted several times into methylene chloride (25 ml. total), and the extract is pressure-filtered and evaporated to dryness in an ignition tube on a steam-bath (Note 8).

(d) *Leucine-2-C^{14}.* Into the tube containing the crude 2-bromo-4-methylvaleric-2-C^{14} acid is distilled 10 ml. of liquid ammonia. The tube is sealed, left at room temperature for 40 hours, then chilled and opened, and the excess ammonia is evaporated. The residue is dissolved in 6 *N* hydrochloric acid and evaporated to dryness. Ethylene oxide is passed for 20 minutes through a filtered solution of the leucine-2-C^{14} hydrochloride in 95% alcohol. The precipitate is collected and recrystallized

three times from water, using decolorizing charcoal twice. The yield is 0.3086 g., and an additional 0.1214 g. is obtained from the mother liquors, making the total yield 52%. Two-dimensional paper chromatography and radioautography show the presence of only one isotopic compound (Note 9).

B. Notes

1. The residue is water-soluble.

2. Micro series reaction vessel (200 ml.), American Instrument Co., Silver Springs, Md.

3. An efficient trap is illustrated in a paper[2] describing the preparation of ethanol-1-C^{14} and -2-C^{14}.

4. The great difference in boiling points of the C_5– and C_{16}– alcohols (131°, 340°, respectively) facilitates the easy isolation of the labeled alcohol in excellent yield and relatively high purity. The alcohol may be prepared in excellent yield by a one-step reduction of the starting acid with lithium aluminum hydride. The latter method,[3,4] however, introduces impurities derived from the solvents that are difficult to remove.

5. The conversion of isopentyl bromide to the acid by carbonation of the Grignard reagent at –25° gives only 40% yields.

6. The yields have been as high as 65% in trial experiments. The purity and identity of the product have been checked by equivalent weight determination and one-dimensional paper chromatography in water-ammonia-propanol solutions.

7. The trap is connected by way of a stopcock and semi-ball joint to a 60-ml. bromination flask equipped with an addition tube and a low-temperature condenser; see Figure II, 9.

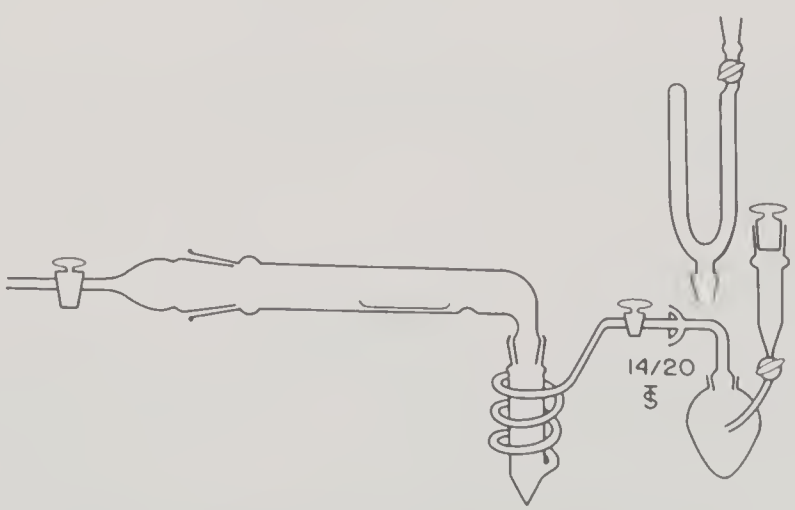


Fig. II, 9. Acid generator and bromination flask (H. Hauptmann, P. T. Adams and B. M. Tolbert). The low temperature reflux condenser is detached.

8. The last traces of solvent are removed effectively by use of the water pump at room temperature.

9. A degradation of C^{14}-leucine is described by Reed.[5]

C. Other Preparations

Leucine-1-C^{14} has been prepared (42% yield) by the same procedure from the 2-bromo-4-methylvaleric-1-C^{14} acid derived from 1.93 g. of sodium 4-methylvalerate-1-C^{14}.

[1]H. Adkins, *Organic Syntheses,* Coll. Vol. II, Wiley, New York, 1943, p. 144.

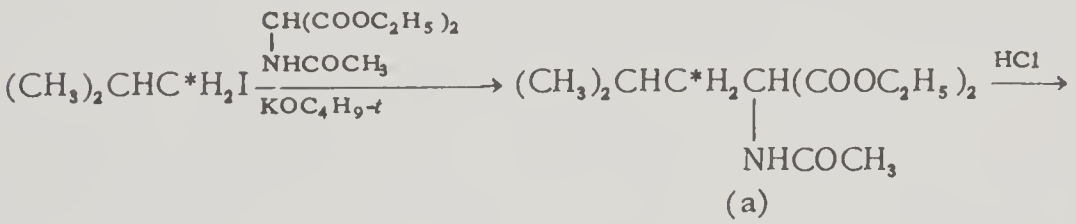
[2]B. M. Tolbert, F. Christenson, F. N. Chang and P. P. T. Sah, J. Org. Chem., *14,* 525 (1949).

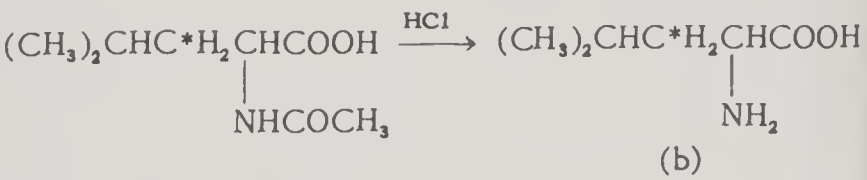
[3]J. D. Cox, H. W. Turner and R. J. Warne, J. Chem. Soc., *1950,* 3167.

[4]R. Ostwald, P. Adams and B. M. Tolbert, J. Am. Chem. Soc., *74,* 2425 (1952).

[5]D. J. Reed, B. E. Christensen, V. H. Cheldelin and C. H. Wang, J. Am. Chem. Soc., *76,* 5574 (1954).

LEUCINE-3-C^{14} HYDROCHLORIDE





R. Ostwald, Atomic Energy Commission Report, UCRL-2154; Nuc. Sci. Abstracts, 7, 3404 (1953).

A. Procedure

(a) *Ethyl Acetamido(2-methylpropyl-1-C^{14})malonate, (Ethyl 2-Acetamido-2-carbethoxy-4-methylvalerate-3-C^{14}).* 1-Iodo-2-methylpropane-1-C^{14} is condensed with ethyl acetamidomalonate in the presence of potassium *t*-butoxide in the manner described for the preparation of valine-4,4′-C^{14} hydrochloride (Note 1).

(b) *Leucine-3-C^{14} Hydrochloride.* The crude malonic ester is hydrolyzed in the manner described for the preparation of valine-4,4′-C^{14} hydrochloride (Note 2). The crude, over-all radiochemical yield is 42–60%, based on three preparations from a total of 26.6 mmoles of halide (Note 3). Purification by fractional elution from the ion exchange column gives the product, contaminated with about 3% of an unknown ninhydrin negative radioactive compound (Note 4). The contaminant is removed by boiling with carbon and recrystallization from water. The over-all yield of purified product is 66%, 59% on a radiochemical basis (Note 5).

B. Notes

1. The crude condensation product may be crystallized[1] by treatment with a small amount of water. Recrystallization from water raises the melting point to 82–83°.

2. The free acid may be prepared[1] by decolorizing the hydrolysis mixture, adjusting the filtrate to pH 6 with concentrated ammonium hydroxide, and cooling overnight to crystallize. The yield of acid in large-scale experiments, m.p. 278–283° (dec.), is 87% based on the condensation product.

3. The crude reaction mixture contains contaminants: glycine and C^{14}-alanine, C^{14}-2-aminobutyric acid and a ninhydrin negative unknown C^{14}-compound.

4. The contaminant is believed to originate in the ion exchange process.

5. Leucine sublimes at 145–148° and melts with decomposition at 290°(rapid heating, sealed tube). The *N*-benzoyl derivative melts at 138–141°.

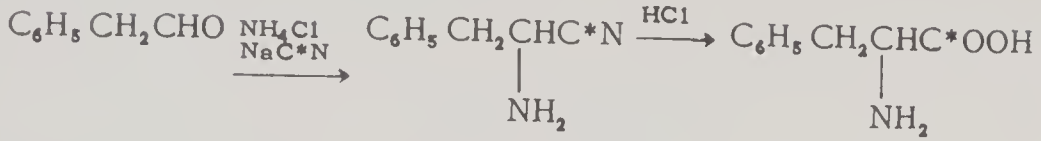
C. Other Preparations

Leucine-4-C^{14} has been prepared[2] in a similar manner, starting with 1-bromo-2-methylpropane-2-C^{14}, through the intermediate ethyl acetamido-(2-methylpropyl-2-C^{14})malonate.

A similar preparation of leucine-3-C^{14} has been reported by Coon and Gurin[2] (42% yield). 1-Bromo-2-methylpropane-1-C^{14} was condensed with ethyl acetamidomalonate in the presence of sodium ethoxide. The crude ethyl acetamido(2-methylpropyl-1-C^{14})malonate was hydrolyzed and decarboxylated by refluxing for 15 hours with constant-boiling hydrobromic acid.

[1]N. F. Albertson and S. Archer, J. Am. Chem. Soc., 67, 308 (1945).
[2]M. J. Coon and S. Gurin, J. Biol. Chem., *180*, 1159 (1949).

3-PHENYLALANINE-1-C^{14}



G. O. Henneberry, W. F. Oliver and B. E. Baker, Can. J. Chem., *29*, 229 (1951).

A. Procedure (Note 1)

A system facilitating the transfer of active materials without contamination by atmospheric carbon dioxide consists of a 500-ml. wide-mouthed flask to which is attached a nitrogen inlet, three test tubes, a small desiccator made from a 29/42 joint, and a fritted-glass filter leading to a small filter flask (Note 2).

To a mixture of crude, freshly-prepared phenylacetaldehyde (Note 3), 0.5575 g. of ammonium chloride, and 1.7 ml. of water cooled to 0° is added slowly 15 ml. of methanol containing the sodium cyanide-C^{14} prepared from 0.1411 g. of barium carbonate-C^{14} (Note 4), followed by 0.5375 g. of sodium cyanide in 6 ml. of 50% methanol. The mixture is stored at room temperature for 18 hours; then 1 ml. of saturated sodium hydroxide solution is added, and the methanol is evaporated under reduced pressure. The residue is extracted into ether solution (Note 5), which is then acidified with 10 ml. of 30 vol.% hydrochloric acid, and the solvent is distilled into alkali (Note 6). The gummy residue is extracted with three 10-ml. portions of boiling 3 *N* hydrochloric acid, and the combined solutions are evaporated to dryness under reduced pressure. The residue is treated at room temperature with fuming hydrochloric acid to remove the last traces of a gummy residue (Note 7). The solution of phenylalanine (2 ml.) is diluted with 5 ml. of ethanol, the pH is adjusted to 5.96 with alcoholic ammonia, the the mixture is stored at 0° to crystallize. The product is collected, and the mother liquor is decolorized with 0.1 g. of Norit A, then concentrated to 0.5 ml., diluted with 1.5 ml. of ethanol and cooled to crystallize a second crop. The combined crude product is recrystallized from alcohol; yield 0.3047 g. The radiochemical yield is 27.6%, based on sodium carbonate-C^{14} (Note 8).

B. Notes

1. The procedure is a modification of the method of Hickinbottom.[1] No product is obtained by the method of Kendall and McKenzie,[2] starting with phenylacetaldehyde.

2. Directions and a diagram of the simple system are given by Henneberry for converting sodium carbonate-C^{14} to sodium cyanide-C^{14}; see

potassium cyanide-C^{14}, Method III. Detailed directions for preparing sodium carbonate-C^{14} are given also by Daudel.[3]

3. A solution of 10 g. of 1,1-dimethoxy-2-phenylethane and 7 ml. of acidic 50% methanol is refluxed for 10 minutes, then evaporated to dryness under reduced pressure.

4. The yield of cyanide is 82 ± 3%.[4]

5. The ether-insoluble salt solution contains 53.8% of the initial activity.

6. The distillate contains 1.2% of the initial activity.

7. The gummy residue contains 10.9% of the initial activity.

8. The mother liquors contain 3.7% of the initial activity. The compound has been used by Fry and Rapoport in studying the mechanism of conversion of α-amino acids to α-acylamino ketones by the Dakin-West reaction; see alanine-1-C^{14}.

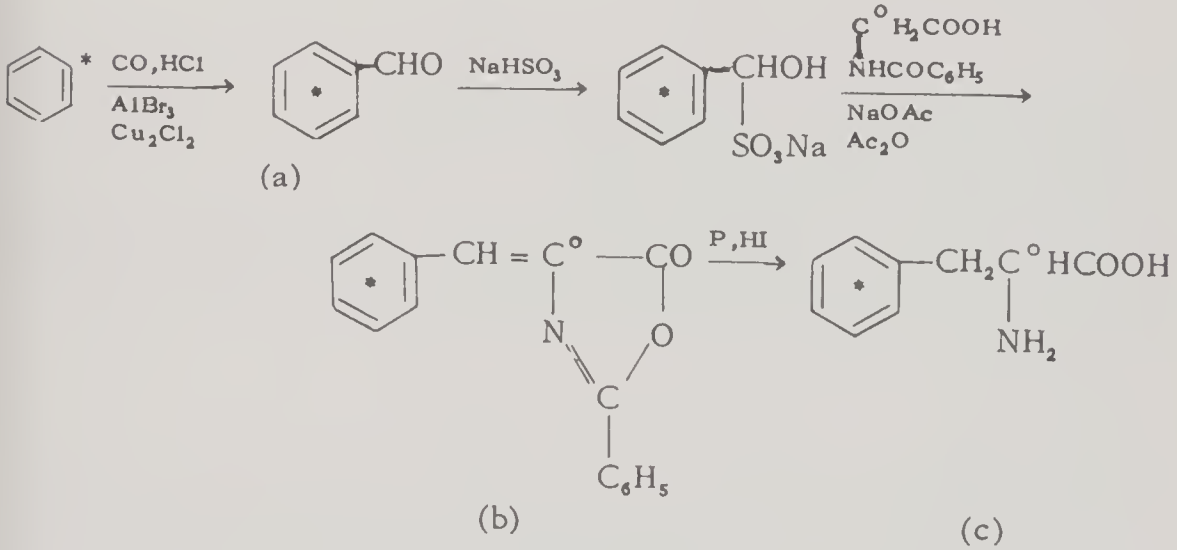
[1]W. J. Hickinbottom, *Reactions of Organic Compounds*, Longmans, Green and Co., Ltd., London, 1936, p. 127.

[2]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 21

[3]P. Daudel and R. Muxart, Bull. soc. chim. France, (5), *18*, 274 (1951).

[4]G. O. Henneberry and B. E. Baker, Can. J. Research, *28B*, 345 (1950).

3-PHENYL-$C^{14}_{1/4}$-ALANINE-2-C^{13}



A. B. Lerner, J. Biol. Chem., *181*, 281 (1949).

A. Procedure

(a) *Benzaldehyde-1,2,3,4-*$C^{14}_{1/4}$ (Note 1). To an ice-cooled solution of 3.187 g. of benzene-C^{14}_1 and 10 ml. of anhydrous cyclohexane, in a 50-ml. centrifuge cone, is added 0.6 g. of anhydrous cuprous chloride[1] and 11.5

g. of anhydrous aluminum bromide. A 2:1 mixture[1] of anhydrous carbon monoxide and hydrogen chloride is bubbled through the cold vigorously stirred reaction mixture for 30 minutes. The mixture is poured slowly onto cracked ice, and the aqueous phase is extracted with two 10-ml. portions of 1:3 benzene-cyclohexane. The combined extracts are treated with an excess of alcoholic sodium bisulfite, and the precipitated complex is filtered off, washed with benzene and air-dried. The yield of bisulfite complex is 3.4 g., 40% (Note 2).

(b) *4-Benzylidene-1,2,3,4-$C^{14}_{1/4}$-2-phenyl-2-oxazolin-5-one-4-C^{13}* (Note 3). A mixture of 3.4 g. of benzaldehyde-1,2,3,4-$C^{14}_{1/4}$ bisulfite, 2.91 g. of hippuric-α-C^{13} acid, 1.35 g. of freshly fused sodium acetate, 9.2 ml. of acetic anhydride, 1.0 ml. of glacial acetic acid and 20 ml. of anhydrous benzene is refluxed for 6 hours. The mixture is cooled, and the crystalline product is collected and washed with a few ml. of boiling water; yield 2.19 g.

(c) *3-Phenyl-$C^{14}_{1/4}$-alanine-2-C^{13}*. The azlactone is treated with red phosphorus and hydriodic acid according to the procedure of Gillespie and Snyder;[2,7] also see Calvin.[3] The yield is 0.760 g. 70% (Note 4).

B. Notes

1. The procedure is adapted from the method of Reformatsky;[4] also see the Gatterman-Koch reaction.[1,5]

2. The yield in trial runs was usually about 60%. The benzene-cyclohexane layer, after separation from the alcoholic bisulfite layer, is dried and distilled for recovery of unreacted benzene-C^{14}.

3. The procedure is adapted from the method of Buck and Ide;[6] also see a review of azlactones by Carter.[7]

4. The DL-form is resolved by the procedure of du Vigneaud and Meyer.[8] Both the L- and the D-forms melt at 284–288° (dec.), and resolution is complete. Degradations are described by Gilvarg.[9]

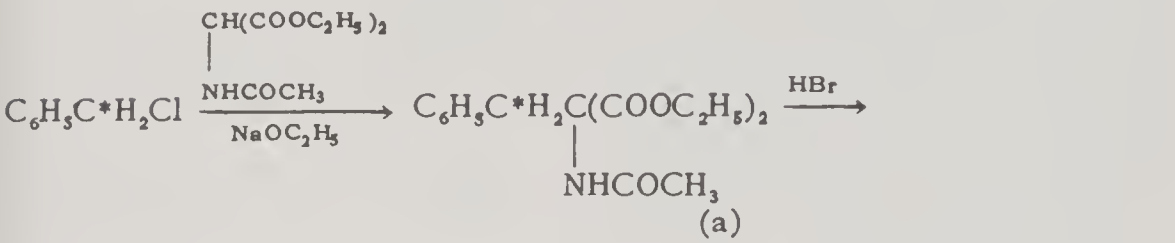
C. Other Preparations

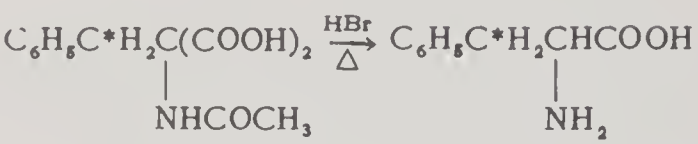
By a similar procedure from benzaldehyde and hippuric-C^{14}-α-C^{14} acid are prepared[3,10,11] 4-benzylidene-2-phenyl-2-oxazolin-5-one-4,5-C^{14} (72% yield) and 3-phenylalanine-1,2-C^{14}_2 (50% yield based on hippuric acid).

[1] *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 583.
[2] *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 489.
[3] M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert, P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 223.
[4] S. Reformatsky, J. Russ. Phys. Chem. Soc., 33, 154 (1901).
[5] *Organic Reactions*, Vol. V, Wiley, New York, 1949, Chapter 6.
[6] *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 55, 490.

[7]*Organic Reactions*, Vol. III, Wiley, New York, 1946, p. 198.
[8]V. du Vigneaud and C. E. Meyer, J. Biol. Chem., *98*, 295 (1932).
[9]C. Gilvarg and K. Bloch, J. Biol. Chem., *199*, 689 (1952).
[10]S. Gurin and A. M. Delluva, J. Biol. Chem., *170*, 545 (1947).
[11]B. Schepartz and S. Gurin, J. Biol. Chem., *180*, 663 (1949).

3-PHENYLALANINE-3-C^{14}





(b)

J. C. Reid, Atomic Energy Commission Report, AECU-216; Nuc. Sci. Abstracts, *2*, 2091 (1949).

A. Procedure (Note 1)

(a) *Ethyl Acetamido(benzyl-α-C^{14})malonate.* To a solution of 1.23 g. (5.66 mmoles) of vacuum-dried ethyl acetamidomalonate and 5.00 mmoles of sodium ethoxide in 7.08 ml. of absolute alcohol (Note 2), under nitrogen in a 15 × 150-mm. heavy-walled Pyrex ignition tube, is added a solution of 0.539 g. (4.24 mmoles) of α-chlorotoluene-α-C^{14}. The tube is capped, cooled with liquid nitrogen and sealed off at a constriction. The mixture is heated at 100° for 2.5 hours; then the sodium chloride is separated by centrifugation, and the colorless solution is evaporated at room temperature with an air stream. An additional small amount of salt is removed by extracting the residue with 5 ml. of hot benzene; evaporation leaves 1.470 g. of colorless crude product (Note 3).

(b) *3-Phenylalanine-3-C^{14}.* The crude condensation product is hydrolyzed and decarboxylated by refluxing under nitrogen for 8 hours with 16.9 ml. of hydrobromic acid (saturated at room temperature). The solution then is diluted with 5 ml. of water, washed with two 5-ml. portions of benzene and evaporated under vacuum. The residue is dissolved in 5 ml. of water and decolorized with carbon, and the filtrate is concentrated on a steam-bath to incipient crystallization. The hot solution is ad-

justed to pH 6 with 6 *N* ammonium hydroxide and stored in a refrigerator overnight; yield 0.437 g. (55.5%), m.p. 219–222° (dec.) (Note 4).

B. Notes

1. The procedure is adapted from the modification of the Sörenson method of preparing α-amino acids described by Albertson;[1] see aspartic-3-C^{14} acid hydrochloride. The alternative methods, involving the condensation of benzaldehyde-C^{14} with some derivative of glycine (acetylglycine, hydantoin, acetylthiohydantoin, or hippuric acid), followed by reduction and hydrolysis of the condensation product, give yields of less than 29% based on benzoic-C^{14} acid.

2. The solution is an aliquot of one assayed by titration. The tube is charged in a nitrogen-filled dry-box.

3. The crude product (113%), containing the excess ethyl acetamidomalonate, is used without further purification. After recrystallization from water, the product melts at 104–104.2°. Purification is also accomplished by crystallization from chloroform-light petroleum.[2]

4. The yield is 40% based on benzoic acid. Additional product (about 0.070 g., based on solubility) may be obtained only by the use of carrier, due to the presence of glycine.

C. Other Preparations

The preparation of ethyl acetamido(benzyl-α-C^{14})malonate and 3-phenylalanine-3-C^{14} by essentially this procedure has been described by Bergel;[2] yield 75% and 52.5%, respectively, based on benzoic acid. The molar specific activity was the same as that of the starting material, and the product exhibited the same R_f value as an authentic sample when chromatographed on buffered paper with 1:1 benzyl alcohol-butyl alcohol.[3]

The preparation of 3-phenyl-1,3,5-C_3^{14}-alanine has been reported by Schepartz;[4] yield 40% based on α-bromotoluene-1,3,5-C_3^{14}.

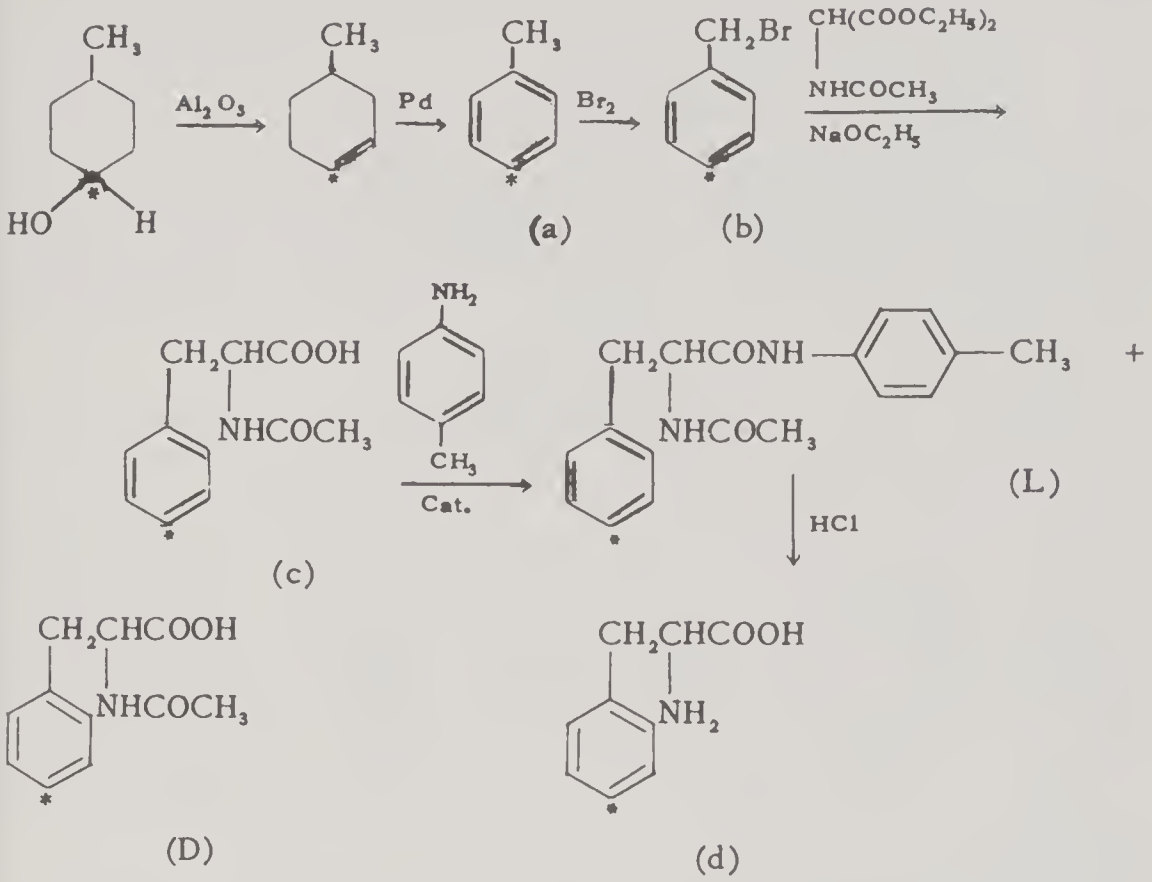
[1] N. F. Albertson and S. Archer, J. Am. Chem. Soc., 67, 308 (1945); H. R. Snyder, J. F. Shekleton and C. D. Lewis, J. Am. Chem. Soc., 67, 310 (1945).

[2] F. Bergel, V. C. E. Burnop and J. A. Stock, J. Chem. Soc., *1955*, 1223.

[3] E. F. McFarren, Anal. Chem., *23*, 168 (1951).

[4] B. Schepartz and S. Gurin, J. Biol. Chem., *180*, 663 (1949).

3-L-PHENYLALANINE-4-C^{14}



R. Dische and D. Rittenberg, J. Biol. Chem., *211*, 199 (1954).

A. Procedure

(a) *Toluene-4-C^{14}.* 4-Methylcyclohexanol-1-C^{14} is dehydrated over aluminum oxide at 375° (see methyl-C^{14}-cyclopentane and 1-methylcyclohexene-1-C^{14}). The 4-methylcyclohexene-1-C^{14} formed is then dehydrogenated over 5% palladium-on-aluminum oxide at 375° (see toluene-1-C^{14}), giving toluene in nearly quantitative yield (Note 1).

(b) *α-Bromotoluene-4-C^{14}, (Benzyl-4-C^{14} Bromide).* Crude toluene-4-C^{14} (50 mmoles) is photo-brominated in the side chain by the general procedure of Sampey; see α-bromotoluene-1,3,5-C_3^{14}; yield 70%, b.p. 90° (16 mm.).

(c) *N-Acetyl-3-phenylalanine-4-C^{14}.* The isotopic synthesis is made with 6.85 g. (40 mmoles) of benzyl-4-C^{14} bromide as described for 3-phenylalanine-3-C^{14}. The crude product (5.85 g.) is purified by recrystallization from alcohol-water; yield 5.00 g. (60%), m.p. 149–150° (cor.).

(d) *3-L-Phenylalanine-4-C^{14}.* The resolution of 5.00 g. of *N*-acetyl-3-phenylalanine-4-C^{14} is performed by the enzymatic (Note 2) method of Huang,[1] which follows; yield 1.580 g. (75%), $[\alpha]_D^{27}$ −34.5° (*c*, 1 in water). The radiochemical yield is 2.24% based on barium carbonate.

In a solution of 9 g. of L-cysteine hydrochloride in 2 l. of 0.5 *M* acetic acid-0.5 *M* sodium acetate buffer is dissolved 155.5 g. (0.75 mole) of *N*-acetyl-3-phenylalanine with warming to 50°. The solution is mixed with 80 g. (0.75 mole) of *p*-toluidine and 150 ml. of papain solution (Note 3), then is diluted to 3 l. with the buffer and heated at 40° for 7 days (pH 4.6). The mixture is stored at 5° for 2 hours, and the crude *N*-acetyl-3-L-phenylalanine *p*-toluidide is collected, washed with 1 l. of water and air-dried (Note 4); yield 102–106 g. (92–95%), m.p. 215–217°. Purification is accomplished by recrystallization from 1.25 l. of hot 96% alcohol and washing of the crystals with 500 ml. of cold alcohol; yield 86–89%, m.p. 219°, $[\alpha]_D^{25}$ +35 ± 1° (*c*, 4 in pyridine).

A mixture of 86 g. of *N*-acetyl-3-L-phenylalanine *p*-toluidide and 1100 ml. of 20% hydrochloric acid is refluxed for 16 hours, then evaporated to dryness under reduced pressure. To a solution of the residue in 250 ml. of water is added 450 ml. of concentrated ammonium hydroxide, and the mixture is stored at 5° for 2 hours. The *p*-toluidine is filtered off and washed with cold water, and the combined solutions are washed with two 300-ml. portions of chloroform. The aqueous solution is concentrated to 500 ml. by boiling, then stored at 5° for 2 hours, and the product is collected, washed with 100 ml. of water, then 40 ml. of 96% alcohol, and air-dried. The yield of 28 g. is raised to 38 g. (82%) by concentrating the mother liquors.

(e) *3-D-Phenylalanine-4-C*^{14}. The mother liquor from the preparation of *N*-acetyl-3-L-phenyl-4-C^{14} *p*-toluidide is acidified to Congo red and continuously extracted with ether for 18 hours. The extract is evaporated, the residue is dissolved in 8 ml. of concentrated ammonium hydroxide, and the excess ammonia is removed on a steam-bath. The ammonium salt of *N*-acetyl-3-D-phenylalanine-4-C^{14}, precipitated by adding acetone, is collected and washed with cold acetone. The *N*-acetyl-3-D-phenylalanine-4-C^{14} is crystallized by chilling an acidified (Congo red) solution of the salt in 25 ml. of water; yield 2.240 g. (89.6%), m.p. 177–177.6° (cor.) (Note 5).

3-D-Phenylalanine-4-C^{14} is isolated in 77% yield, following hydrolysis and recrystallization as in (d).

B. Notes

1. The dehydrogenation method is that of Zelinsky,[2] who prepared the palladium-black by reduction of palladium ammonium chloride with formic acid in alkali; also see Mozingo.[3]

2. The resolution of optically racemic amino acids by asymmetric enzymatic hydrolysis of amides, esters and *N*-acyl derivatives is discussed by Levintow.[4]

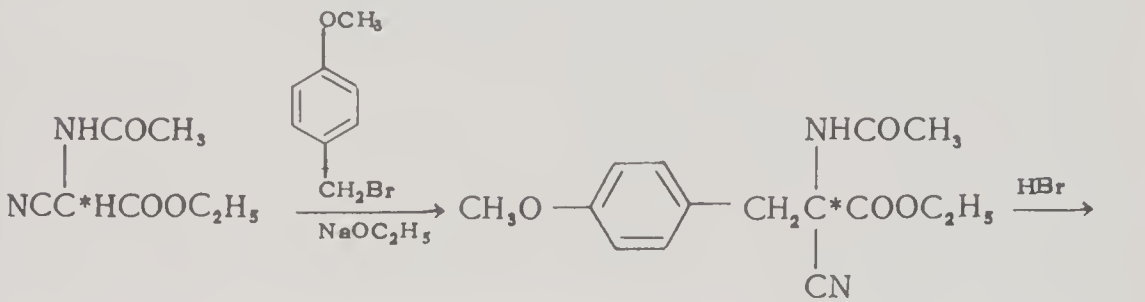
3. A mixture of 30 g. of finely ground papain (Wallerstein) and 150 ml. of water is stirred for 3 hours at 5°, then centrifuged, and the supernatant solution is separated.

4. The mother liquor is saved for isolation of the D-isomer.

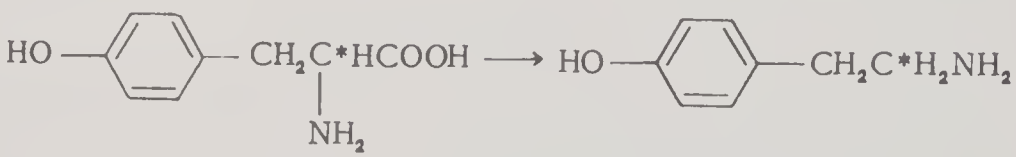
5. $[\alpha]_D^{25} -39°$ (*c*, 2 in methanol), $[\alpha]_D^{25} -46°$ (*c*, 2 in ethanol).

[1]H. T. Huang and C. Niemann, J. Am. Chem. Soc., 73, 475 (1951).
[2]N. Zelinsky, Ber., 44, 3121 (1911).
[3] *Organic Syntheses*, Vol. 26, Wiley, New York, 1946, p. 77.
[4]L. Levintow, V. E. Price and J. P. Greenstein, J. Biol. Chem., *184*, 55 (1950).

TYROSINE-α-C^{14}



(a)



(b)

M. Fields, D. E. Walz and S. Rothchild, J. Am. Chem. Soc., 73, 1000 (1951).

A. Procedure (Note 1)

(a) *Ethyl Acetamidocyano*(p-*methoxybenzyl*)*acetate-2-*C^{14}. A solution of 0.119 g. of sodium in 5 ml. of absolute ethanol is added to a solution of 0.850 g. of ethyl acetamidocyanoacetate-2-C^{14} and 5 ml. of absolute alcohol in a 100-ml. three-necked flask equipped with a mercury-sealed stirrer, reflux condenser and dropping funnel. *p*-Methoxybenzyl bromide[1] (0.79 ml., d. 1.41) is added dropwise with cooling; the mixture is stirred for 2 hours at room temperature, then diluted with 10 ml. of water, and the product is collected; yield 1.39 g. (96%), m.p. 169.5–170.5° (Note 2).

(b) *Tyrosine-*α*-*C^{14}, [*3-*(p-*Hydroxyphenyl*)*alanine-2-*C^{14}]. A mixture of 1.39 g. of ethyl acetamidocyano(*p*-methoxybenzyl)acetate-2-C^{14} and 15 ml. of 48% hydrobromic acid is refluxed for 4 hours (Note 3). The excess acid is vacuum-distilled, and the residue is dissolved in 5 ml. of water and decolorized with charcoal. A slight excess of ammonium hydroxide and then a slight excess of acetic acid are added, and the product is

collected, washed with water and alcohol and dried; yield 0.827 g. (95%), m.p. (evacuated tube) 303–304° (dec.) (Note 4).

B. Notes

1. This method is general for preparing α-amino acids.[2]

2. A sample recrystallized from dilute alcohol melts at 170–171°. Using the chloro- starting material reduced the yield to 85%. Shulgin[3] has reported a 70% yield for this step with the bromide.

3. The same yield is obtained by hydrolysis with concentrated hydrochloric acid in a sealed tube at 160° for 3 hours.

4. Dibenzoyltyrosine melts at 208–209°, after recrystallization from glacial acetic acid; see 3-(*p*-hydroxyphenyl)alanine-1-C^{14}. Melting points are usually more characteristic than decomposition points.

The preparation of 4-hydroxyphenethylamine-α-C^{14} by thermal decarboxylation[4] of tyrosine-α-C^{14} accomplished by heating at 260–265° with an equal mixture of diphenylmethane and diphenylamine, has been reported by Schayer.[5] The product was purified by vacuum sublimation and converted to the picrate,[6] m.p. 201°. Pure tyramine crystallized from benzene or alcohol melts at 164–165°; the hydrochloride melts at 269°. The yield is good.

C. Other Preparations

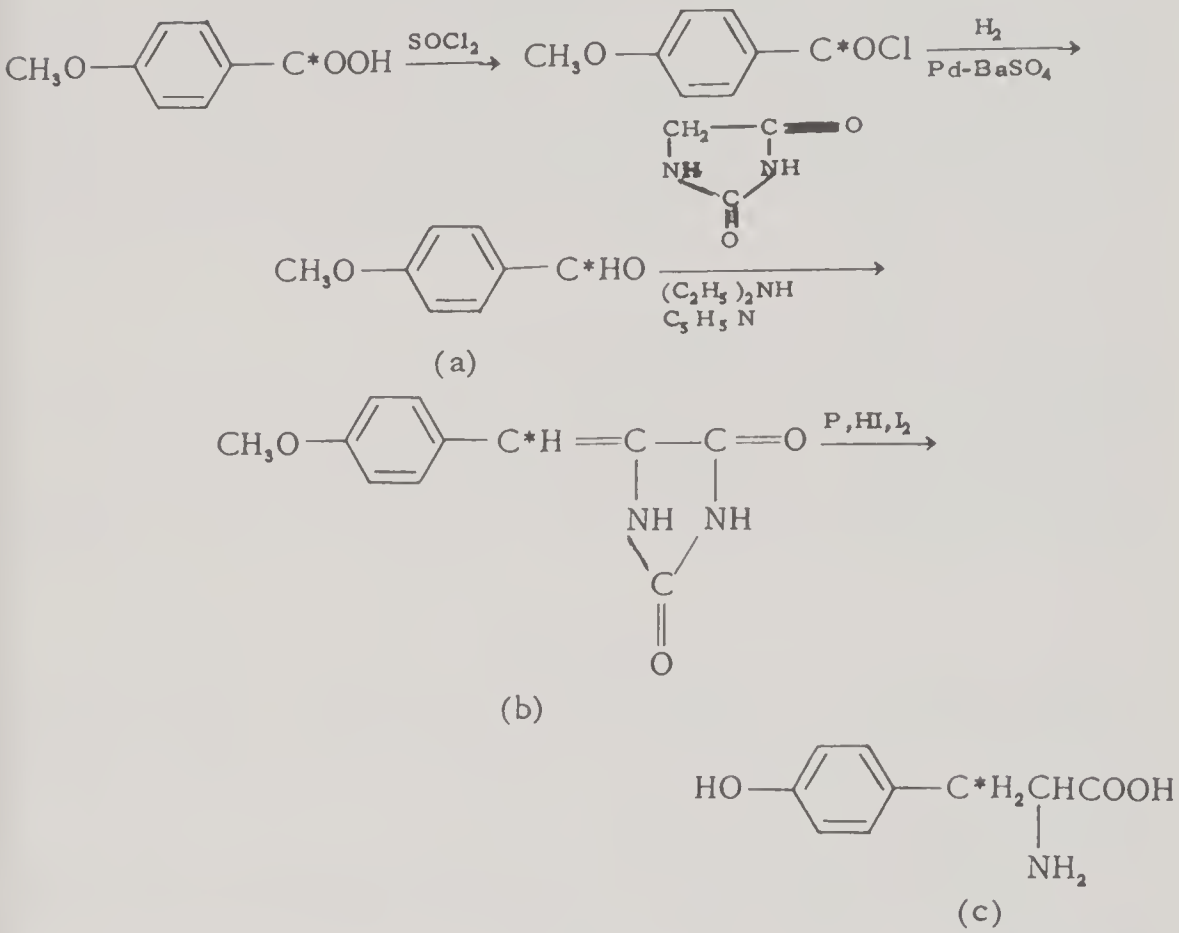
Tyrosine-α-C^{14} has been prepared by Leete[7] by the method described; yield 50.3% based on sodium acetate.

By the same procedure starting with benzyl chloride are prepared[8] ethyl 2-acetamido-2-cyano-3-phenylpropionate-2-C^{14} and 3-phenylalanine-2-C^{14} in 80–85% yield.

A new synthesis of 3-(2,5-dihydroxyphenyl)alanine has been developed and adapted to the isotopic scale by Shulgin.[4] 2,5-Dimethoxybenzyl bromide was condensed with ethyl sodiumacetamidomalonate (yield 75%), and the product was hydrolyzed and decarboxylated in hydriodic-acetic acid (yield 65%). In a similar manner, ethyl 2-acetamido-2-(*p*-methoxybenzyl)malonate was prepared (60% yield) and converted to tyrosine (80% yield). Paper chromatography gave a single ninhydrin-positive spot of the correct R_f value.

[1]H. Stephen and C. Weizmann, J. Chem. Soc., *1914*, 1152.
[2]N. F. Albertson, J. Am. Chem. Soc., *68*, 450 (1946); J. H. Burckhalter and V. C. Stephens, J. Am. Chem. Soc., *73*, 56 (1951).
[3]A. T. Shulgin and E. M. Gal, J. Chem. Soc., *1953*, 1316.
[4]T. B. Johnson and P. G. Daschavsky, J. Biol. Chem., *62*, 725 (1925).
[5]R. W. Schayer, Proc. Soc. Exptl. Biol. Med., *84*, 60 (1953).
[6]G. Barger, J. Chem. Soc., *95*, 1128 (1909).
[7]E. Leete and L. Marion, Can. J. Chem., *31*, 127 (1953).
[8]M. Fields, S. Rothchild, J. Gibbs and D. Walz, Nucleonics, 7(3), 63 (1950).

TYROSINE-β-C^{14}
3-(*p*-Hydroxyphenyl)alanine-3-C^{14}



J. C. Reid and H. B. Jones, J. Biol. Chem., *174*, 427 (1948); J. C. Reid, Science, *105*, 208 (1947).

A. Procedure

(a) p-*Anisaldehyde-*C^{14} (Note 1). A mixture of 0.64 g. (4.2 mmoles) of *p*-anisic-C^{14} acid and 5 ml. of thionyl chloride (Note 2) is refluxed for 5 hours. The excess reagent is removed under vacuum, and the residual crude *p*-anisoyl-C^{14} chloride is dissolved in 7 ml. of pure, dry xylene containing 0.003 ml. of quinoline-sulfur poison and 0.05 g. of 5% palladium-barium sulfate catalyst (Note 3). Purified hydrogen (Note 4) is admitted below the surface of the violently stirred refluxing solution (Note 5) for a period of 4 hours. The solution is centrifuged to remove the catalyst and evaporated to dryness under vacuum; yield 73% or more (3.08 mmoles, Note 6).

(b) 5-(p-*Methoxybenzylidene-*C^{14}*)hydantoin.* A mixture of the crude *p*-anisaldehyde-C^{14}, 0.55 g. (5.5 mmoles) of hydantoin, 0.7 ml. of dry diethylamine and 1.2 ml. of dry pyridine is heated at 100° for 72 hours in a sealed, heavy-wall, Pyrex tube. The reaction mixture is evaporated at room temperature and then for 1.5 hours at 100°, all under vacuum.

The residue is washed with three 2-ml. portions of hot water and dried at 90°; yield 0.60 g., 93% based on anisaldehyde (Note 7).

(c) *Tyrosine-β-C^{14}* (Note 8). A mixture of the crude hydantoin, 0.19 g. of red phosphorus and 2.4 ml. of redistilled hydriodic acid (d. 1.7) is refluxed for 5 hours; then 0.63 g. of iodine is added, and refluxing is continued for 5.5 hours. The filtered solution is evaporated under vacuum, the last of the acid being removed by evaporation of 5 ml. of added water. The residue is dissolved in 5 ml. of water, and the solution is diluted with water (25 ml.) until no additional turbidity results. The solution is clarified by centrifugation, then adjusted to pH 5.7 with concentrated ammonia, filtered, evaporated, and dried at 100° for 15 minutes under vacuum. The residue is washed with water and alcohol, then dried at 90°; yield 0.177 g. (36% based on the hydantoin).

L-Tyrosine-β-C^{14} is prepared[1] by diluting the racemic form with 10 parts of L-tyrosine and recrystallizing the product from water to constant specific activity (Note 9).

B. Notes

1. The procedure is an adaptation of the Rosenmund reduction method described in detail by Hershberg and Cason.[2] A review of the Rosenmund reduction is presented by Mosettig and Mozingo,[3] and a diagram of the apparatus is presented by Calvin.[4]

2. The thionyl chloride (50 ml.) is purified by distillation with the use of a fractionating column and a calcium chloride tube, first from quinoline (10 ml.), then from boiled linseed oil (20 ml.).

3. The catalyst poison is prepared by refluxing 1 g. of sulfur with 6 g. of quinoline for 5 hours, then diluting the dark liquid to 70 ml. with pure xylene. The catalyst may be prepared according to Mozingo.[5]

4. Hydrogen is purified by passage through wash bottles containing Fieser's solution[6] and saturated lead acetate solution, and then is dried over Drierite.

5. The air condenser consists of a tube (20 cm. × 11 mm.), the upper 5 cm. of which is packed with Drierite. The exhaust is attached to a gas disperser in a trap containing sodium hydroxide solution, which serves to observe the evolution of hydrogen chloride. As hydrogen absorption occurs chiefly at the rapidly agitated surface, use of a sealed, magnetic-induction stirrer is recommended.

6. Rosenmund reported a yield of 81%; the figure of 73% was obtained by weighing the 2,4-dinitrophenylhydrazone. The isotopic product was not isolated, but the evolved hydrogen chloride was equivalent to 88% of the *p*-anisic acid.

7. The yield in trial runs was about 90%. 5-(*p*-Methoxybenzylidene) hydantoin crystallized from alcohol melts[7] at 243–244°.

8. The procedure is that of Wheeler and Hoffman.[7] Phosphorus and hydriodic acid reduce the double bond, evolve methyl iodide with opening of the hydantoin ring, and hydrolyze the urea group.

9. The measurement of radioactivity is much more sensitive than optical methods for detecting the presence of minute amounts of contaminating isomers. The method of resolution appears to be generally applicable to isotopic α-amino acids or other racemic compounds; see cystine-3,3′-C_2^{14}. A degradation has been outlined by Thomas[8] and Gilvarg,[9] and described in detail by Reio.[10]

C. Other Preparations

Veratraldehyde-C^{14}, (3,4-dimethoxybenzaldehyde-C^{14}), is prepared[11,12] by the sequence: veratric-C^{14} acid, veratroyl-C^{14} chloride; yield 58% based on the acid chloride. The evolution of hydrogen chloride is equivalent to 73% of the veratric acid.[12] Swan[12] has reported the desirability of distilling the acid chloride prior to reduction.

The preparation of 4-hydroxy-3-methoxybenzaldehyde-C^{14}, (C^{14}-vanillin), by a similar procedure has been reported by Brown[13] and Freudenberg[14] (25% yield based on barium carbonate), and described in detail by Kratzl.[15] 4-Benzyloxy-*m*-anisoyl-C^{14} chloride was prepared by refluxing 4-benzyloxy-*m*-anisic-C^{14} acid with thionyl chloride for 1.5 hours and was distilled at 0.1 mm. with an air-bath at 160°; yield 75%, m.p. 63°. The simultaneous reduction and hydrogenolysis required 6 hours. The oily residue resulting from evaporation of the reaction solution was refluxed for 1 hour with 6 *N* hydrochloric acid, and the crude product was isolated by ether extraction. Purification was accomplished through the C^{14}-vanillin *m*-nitrophenylhydrazone (yield 67%), which was then decomposed in boiling 2 *N* sodium hydroxide solution by the dropwise addition of 1 *N* mercuric chloride solution, nitrogen being evolved and mercury precipitated. The cooled solution was filtered, neutralized with hydrochloric acid and extracted with ether; recovery of aldehyde was 98% based on the hydrazone.

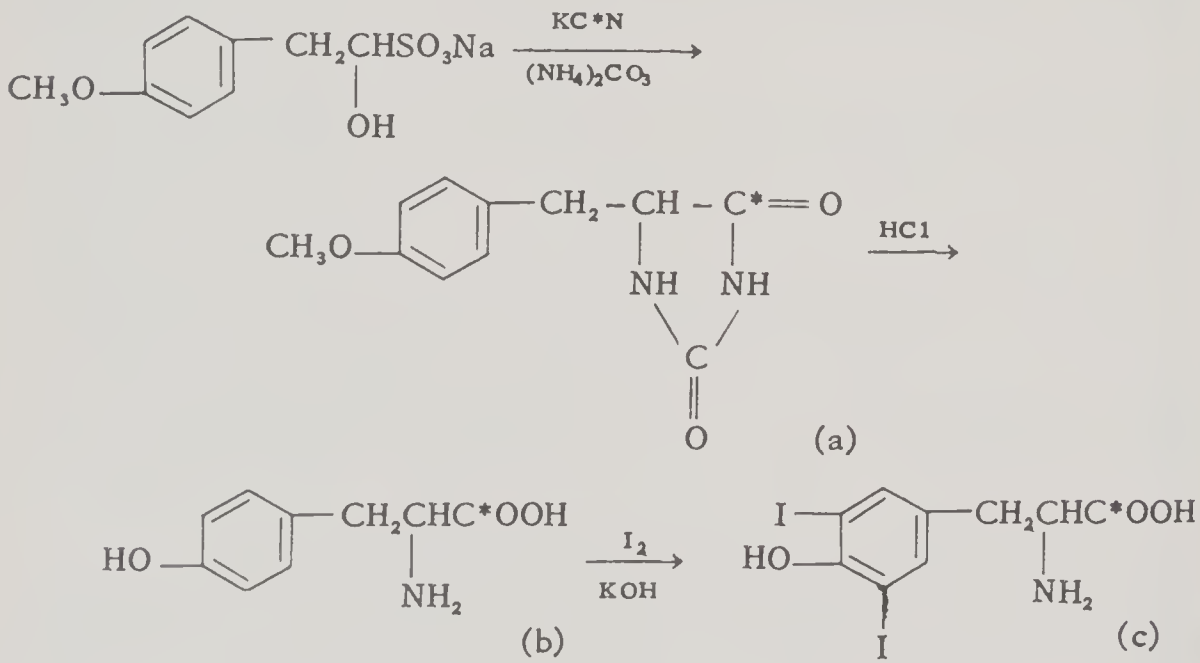
The similar preparation of syringaldehyde-C^{14}, (4-hydroxy-3,5-dimethoxybenzaldehyde-C^{14}), has been outlined by Freudenberg;[16,14] yield 22% based on barium carbonate. 4-Benzyloxy-3,5-dimethoxybenzoic-C^{14} acid was subjected to hydrogenolysis, and the syringic-C^{14} acid was acetylated. Reduction of the 4-acetyl-3,5-dimethoxybenzoyl-C^{14} chloride gave syringaldehyde-C^{14} acetate, which yielded the product upon hydrolysis.

[1]S. Weinhouse and R. H. Millington, J. Biol. Chem., *175*, 997 (1948); J. L. Wood and H. R. Gutmann, J. Biol. Chem., *179*, 535 (1949).

[2]*Organic Syntheses*, Vol. 21, Wiley, New York, 1941, p. 84; K. W. Rosenmund, F. Zetzsche and G. Weiler, Ber., 56, 1481 (1923).

[3]*Organic Reactions*, Vol. IV, Wiley, New York, 1948, Chapter 7.
[4]M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 199.
[5]*Organic Syntheses*, Vol. 26, Wiley, New York, 1946, p. 77.
[6]L. F. Fieser, J. Am. Chem. Soc., *46*, 2639 (1924).
[7]H. L. Wheeler and C. Hoffman, Am. Chem. J., *45*, 368 (1911).
[8]R. C. Thomas, V. H. Cheldelin, B. E. Christensen and C. H. Wang, J. Am. Chem. Soc., *75*, 5554 (1953).
[9]C. Gilvarg and K. Bloch, J. Biol. Chem., *199*, 689 (1952).
[10]L. Reio and G. Ehrensvärd, Arkiv Kemi, *5*, 301 (1953).
[11]M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 119.
[12]G. A. Swan and D. Wright, J. Chem. Soc., *1954*, 381.
[13]S. A. Brown and A. C. Neish, Can. J. Biochem. and Physiol., *33*, 948 (1955).
[14]K. Freudenberg and M. Reichert, Ber., *87*, 1834 (1954).
[15]K. Kratzl and G. Billek, Holzforschung, *7*, 66 (1953); K. Kratzl and E. Kvasnicka, Monatsh. Chem., *83*, 18 (1952); Austr. 168,059.
[16]K. Freudenberg and M. Reichert, Naturwiss., *41*, 229 (1954).

3-(4-HYDROXY-3,5-DIIODOPHENYL)ALANINE-1-C^{14}
(C_1^{14}-Diiodotyrosine)



R. B. Loftfield, J. Am. Chem. Soc., *72*, 2499 (1950).

A. Procedure

(a) *5-(p-Methoxybenzyl)hydantoin-4-*C^{14} (Note 1). The hydrogen cyanide-C^{14} prepared from 1 mmole of barium carbonate-C^{14} is collected in 1.2 mmoles of sodium methoxide and evaporated to dryness in a 12 × 200-mm. Pyrex tube. To the residue is added 0.065 g. of potassium cyanide,

0.600 g. of (*p*-methoxyphenyl)acetaldehyde sodium bisulfite compound (Note 2), 0.600 g. of pulverized ammonium carbonate and 3 ml. of 50% ethanol-water. The sealed tube is heated at 100° for 4 hours, then opened, and the alcohol and ammonium carbonate are removed by warming the mixture to 104°. Hot water (2.0 ml.) is added, and the clear solution is chilled in ice for 2 hours. The product is collected and washed with 2 ml. of water (Note 3); yield 0.330 g. (75%). The product crystallizes from alcohol in colorless leaflets, m.p. 176–179°.

(b) *3-(p-Hydroxyphenyl)alanine-1-C*^{14}, (*C*$_1^{14}$-*Tyrosine*). The 5-(*p*-methoxybenzyl)hydantoin-4-C^{14} (0.240 g.) is heated with 2 ml. of concentrated hydrochloric acid in a sealed tube for 2 hours at 140° to 160° (final). The mixture is diluted with water to 10 ml., heated to boiling and adjusted to pH 6 with ammonium hydroxide and acetic acid. The hot solution is filtered through carbon and cooled (Note 4); yield 0.175 g. (89%), m.p. 314–315° (dec.) (Note 5).

(c) *3-(4-Hydroxy-3,5-diiodophenyl)alanine-1-C*^{14} (Note 6). To a solution of 0.134 g. of 3-(*p*-hydroxyphenyl)alanine-1-C^{14} and 0.146 g. of potassium hydroxide in 30.0 ml. of water (contained in a centrifuge tube under nitrogen) is added 0.408 g. of iodine with stirring. Acetic acid (0.10 ml.) is added after 15 minutes and, after standing for another 15 minutes, the precipitate is filtered by centrifugation and washed with water. The precipitate is mixed with 0.10 ml. of hydrochloric acid in 4.0 ml. of water, and the suspension is filtered through Norit (0.05 g.) into a solution of 0.20 g. of sodium acetate and 1.0 ml. of water. The product slowly separates as buff-colored crystals, which are collected, washed and dried; yield 0.182 g. (57%), m.p. 195° (dec.).

B. Notes

1. The procedure is an adaptation of the Bucherer[1] hydantoin synthesis. The Strecker and Erlenmeyer syntheses were unsatisfactory for the preparation of tyrosine.

2. Directions are given by Loftfield for preparing the aldehyde bisulfite compound by a modification of the directions given by Rosenmund and Domsaft[2] for the Darzens[3] aldehyde synthesis from *p*-anisaldehyde and ethyl chloroacetate. Acidification of the intermediate sodium (*p*-methoxyphenyl)glycidate gives the aldehyde contaminated always with resin. However, decarboxylation of the salt in hot sodium bisulfite solution gives the stable pure product in 56% yield. See 5-(3-hydroxy-4-methoxybenzyl)hydantoin-4-C^{14}.

3. A diagram of a convenient means of filtration employing the centrifuge is presented by Loftfield.

4. Crystallization sometimes does not occur in the case of clear, colorless solutions, if the charcoal treatment is neglected.

5. Dibenzoyltyrosine, m.p. 213-214°, is prepared by treating the product with benzoyl chloride in 2.5% aqueous sodium hydroxide. Directions are given by Clemo[4] for preparing C^{14}-melanin by oxidation of the tyrosin. A systematic degradation of isotopic tyrosine has been described by Baddiley.[5]

6. Oswald[6] has improved the yield of this procedure of Wheeler and Jamieson, by iodinating at 0°. Block and Powell[8] have reported yields of 80–85% by iodinating with iodine monochloride. Wang[9] has reported that the iodination of tyrosine in 20% ethylamine solution is effected in nearly quantitative yield (see C_1^{14}-thyroxine).

C. Other Preparations

Clemo, Duxbury and Swan[4] have prepared 3-(*p*-hydroxyphenyl)alanine-1-C^{14} (23% radiochemical yield) by a modification of the described method. These investigators found it desirable, in the interests of purity in the final product, to hydrolyze the hydantoin by refluxing for 8 hours with strong barium hydroxide solution. After removal of barium as the sulfate, the intermediate 3-(*p*-methoxyphenyl)alanine-1-C^{14} crystallized from the cold concentrated solution at about pH 5; yield 0.190 g. (m.p. 245–250°) from 0.247 g. of the hydantoin. Decarboxylation by treatment with ninhydrin[10] has demonstrated the location of isotope at C-1. This methyl ether was then hydrolyzed to the tyrosine by heating with hydrochloric acid for 1 hour at 120°, in essentially the manner described.

Maimind[11] has described a similar preparation: 5-(*p*-methoxybenzyl)-hydantoin-4-C^{14} (71% yield, m.p. 176–179°) and 3-(*p*-hydroxyphenyl)-alanine-1-C^{14} (81.7% yield, m.p. 262°). The methoxyl group was removed by refluxing for 4 hours with 48% hydrobromic acid.

[1]H. T. Bucherer and W. Steiner, J. prakt. Chem., *140*, 291 (1934); H. T. Bucherer and V. A. Libe, J. prakt. Chem., *141*, 5 (1935).

[2]K. W. Rosenmund and H. Dornsaft, Ber., *52*, 1734 (1919).

[3]G. Darzens, Compt. rend., *139*, 1214 (1904).

[4]G. R. Clemo, F. K. Duxbury and G. A. Swan, J. Chem. Soc., *1952*, 3464.

[5]J. Baddiley, G. Ehrensvärd, E. Klein, L. Reio and E. Saluste, J. Biol. Chem., *183*, 777 (1950).

[6]A. Oswald, Hoppe Seyler's Z. physiol. Chem., *59*, 320 (1909).

[7]H. L. Wheeler and G. S. Jamieson, Am. Chem. J., *33*, 365 (1905).

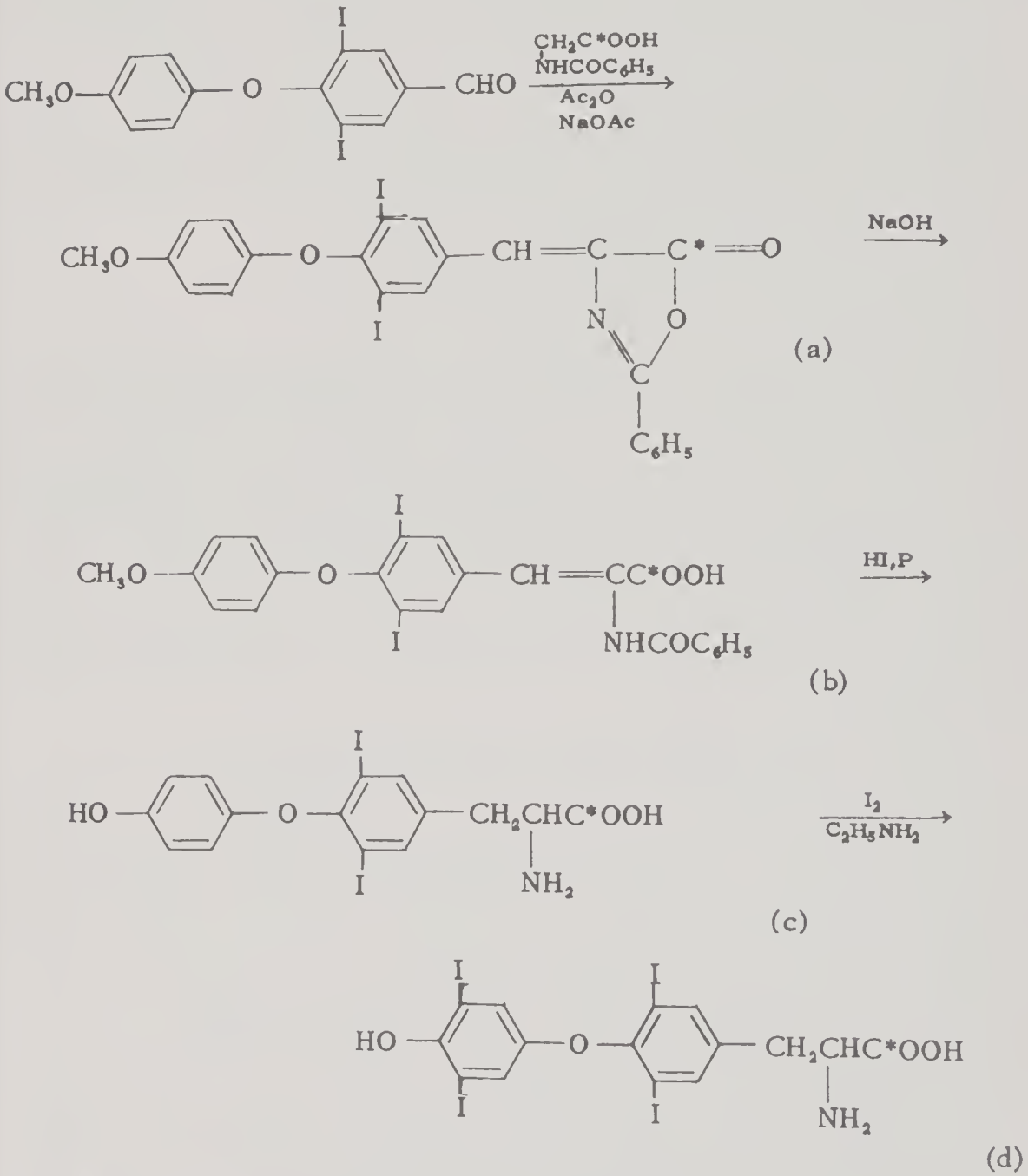
[8]P. Block, Jr., and G. Powell, J. Am. Chem. Soc., *65*, 1430 (1943).

[9]S. C. Wang, J. P. Hummel and T. Winnick, J. Am. Chem. Soc., *74*, 2445 (1952).

[10]D. D. Van Slyke, D. A. MacFadyen and P. Hamilton, J. Biol. Chem., *141*, 671 (1941).

[11]V. I. Maimind, B. V. Takarev and M. M. Shemakin, Doklady Akad. Nauk S.S.S.R., *92*, 81 (1953); Chem. Abstracts, *48*, 10588 (1954).

3-[3,5-DIIODO-4-(3,5-DIIODO-4-HYDROXYPHENOXY)PHENYL]ALANINE-1-C^{14} (C_1^{14}-Thyroxine)



S. C. Wang, J. P. Hummel and T. Winnick, J. Am. Chem. Soc., 74, 2445 (1952).

A. Procedure (Note 1)

(a) *4-[3, 5-Diiodo-4-(p-methoxyphenoxy)benzylidene]-2-phenyl-2-oxazalin-5-one-5-C^{14}.* To an intimate mixture of 0.240 g. (1.34 mmoles) of hippuric-C^{14} acid, 0.663 g. (1.38 mmoles) of 3,5-diiodo-4-(*p*-methoxyphe-

noxy)benzaldehyde (Note 2) and 0.631 g. of freshly fused sodium acetate is added 1.9 ml. of acetic anhydride. The mixture is heated for 15 minutes on a steam-bath, then ground with 1-ml. portions of water, and the solid is collected and dried. The yield of crude azlactone is quantitative. The recrystallized product is obtained in 73% yield, m.p. 211–213°, from glacial acetic acid.

(b) *α-Benzamido-3,5-diiodo-4-(p-methoxyphenoxy)cinnamic-C*^{14} *Acid.* A solution of 0.609 g. of the azlactone, 44 ml. of ethanol, 0.6 g. of sodium hydroxide and 16 ml. of water is boiled for 5 minutes, then acidified with 1.7 ml. of concentrated hydrochloric acid and cooled. The product is collected in 0.5516-g. yield (88.1%), m.p. 237–239° (dec.).

(c) *3-[3,5-Diiodo-4-(p-hydroxyphenoxy)phenyl]alanine-1-C*^{14}, (*C*$_1^{14}$*-3,5-Diiodothyronine*). A mixture of the cinnamic acid, 2.76 ml. of hydriodic acid (d. 1.70), 0.334 g. of red phosphorus and 2.76 ml. of acetic anhydride is refluxed for 1.25 hours. The phosphorus is filtered off and washed with 1 ml. of glacial acetic acid. The combined solutions are evaporated to dryness under reduced pressure; then a few ml. of water are added, and the process is repeated. The residue is treated with 4.4 ml. of boiling water and, after cooling, the solid is collected and washed with ether. The filtrate is washed with two portions of ether, then combined with the first solid and treated, at the boiling point, with 0.7 ml. of concentrated hydrochloric acid. After cooling, the hydrochloride is collected, and the product is liberated by treatment with ammonia; yield 0.3694 g. (83.7%), m.p. 247° (dec.).

(d) *3-[3,5-Diiodo-4-(3, 5-diiodo-4-hydroxyphenoxy)phenyl]alanine-1-C*^{14} (Note 3). To a stirred solution, in a 12 ml. centrifuge tube, containing 0.361 g. of C_1^{14}-diiodothyronine and 3.7 ml. of 20% ethylamine is added a solution of 0.400 g. of potassium iodide, 0.376 g. of iodine and 1.4 ml. of water. The mixture is stirred for 12 minutes, then acidified with 0.3 ml. of glacial acetic acid and treated with 1 ml. of saturated sodium bisulfite solution. The mixture is stored for a few hours in the cold, then treated with 3 ml. of water, and the solid is separated by centrifuging. The residue is washed with a 4.5-ml. and a 3-ml. portion of hot ethanol-2 *N* sodium hydroxide (2:1). The combined supernatant solutions are acidified with 0.3 ml. of acetic acid, and the product is collected in 0.5329-g. yield (99.6%). Purification is effected by dissolution in hot dilute sodium carbonate solution, separation from a slight, colored residue and crystallization of the sodium salt by cooling. The salt is taken up in 2 *N* sodium hydroxide, and the product is reprecipitated by acidification with glacial acetic acid, collected, and washed with water, alcohol and ether; yield 0.3182 g., m.p. 232–234° (Note 4). Degradation studies indicate that there is no isotopic rearrangement (Note 5).

B. Notes

1. The procedure is a modification of the method of Harington.[1]

2. Directions are given by Harington[1] for preparing the starting material.

3. The procedure is adapted from the method of Clayton and Hems.[2]

4. The specific activity is virtually the same as that of the crude product.

5. The acid is decarboxylated at pH 2.5 with ninhydrin by the method described by Van Slyke.[3] The molar specific activity of the barium carbonate-C^{14} formed is the same as that of the amino acid.

C. Other Preparations

3-[3,5-diiodo-4-(3,5-diiodo-4-hydroxyphenoxy)phenyl]alanine-2-C^{14} is prepared[4] similarly from glycine-2-C^{14}.

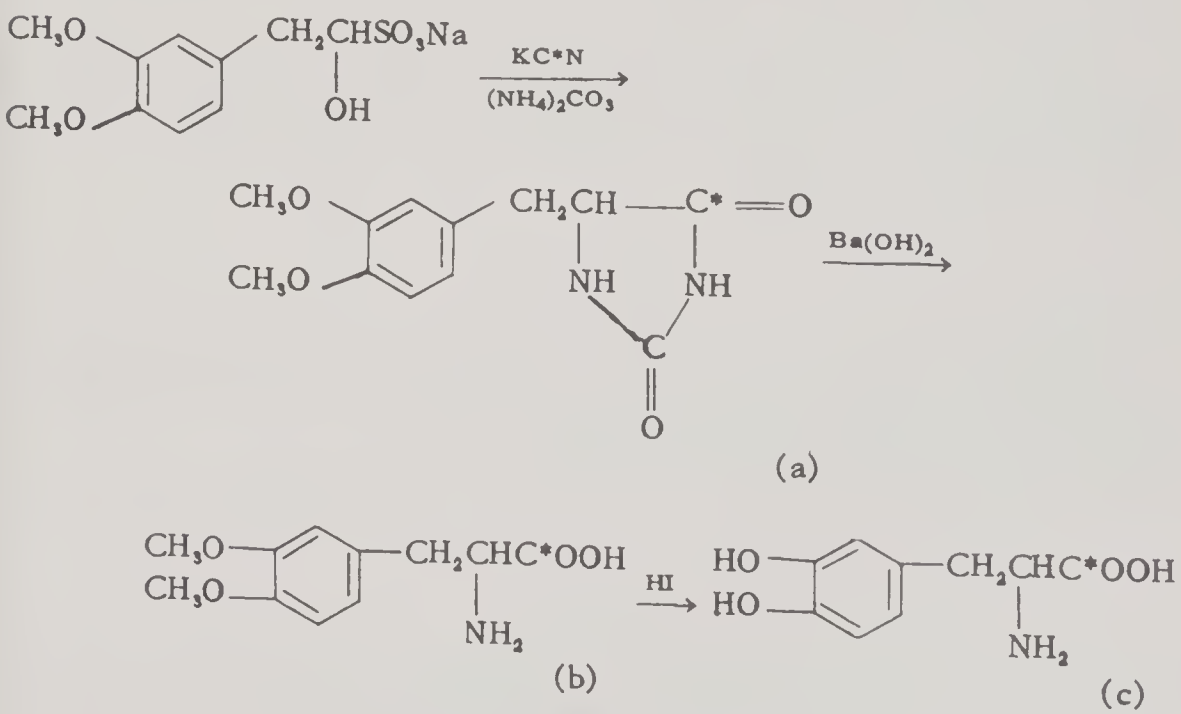
[1]C. R. Harington and G. Barger, Biochem. J., *21*, 169 (1927); C. R. Harington and W. McCartney, Biochem. J., *21*, 852 (1927).

[2]J. C. Clayton and B. A. Hems, J. Chem. Soc., *1950*, 840.

[3]D. D. Van Slyke, D. A. MacFadyen and P. Hamilton, J. Biol. Chem., *141*, 671 (1941).

[4]R. M. Lemmon, Atomic Energy Commission Report, AECD-3504; Nuc. Sci. Abstracts, 7, 5013 (1953).

3-(3,4-DIHYDROXYPHENYL)ALANINE-1-C^{14}



G. R. Clemo, F. K. Duxbury and G. A. Swan, J. Chem. Soc., *1952*, 3464.

A. Procedure

(a) *5-(3,4-Dimethoxybenzyl)hydantoin-4-C*^{14}. A mixture of 0.600 g. of (3,4-dimethoxyphenyl)acetaldehyde sodium bisulfite (Note 1), 0.600 g. of ammonium carbonate, 0.1326 g. of potassium cyanide-C^{14} and 3 ml. of 50% aqueous alcohol is heated in a sealed tube for 4 hours at 100°. The mixture is diluted with 3 ml. of water, then heated for 30 minutes at 105–110° and cooled overnight; yield 0.250 g., m.p. 156–160°.

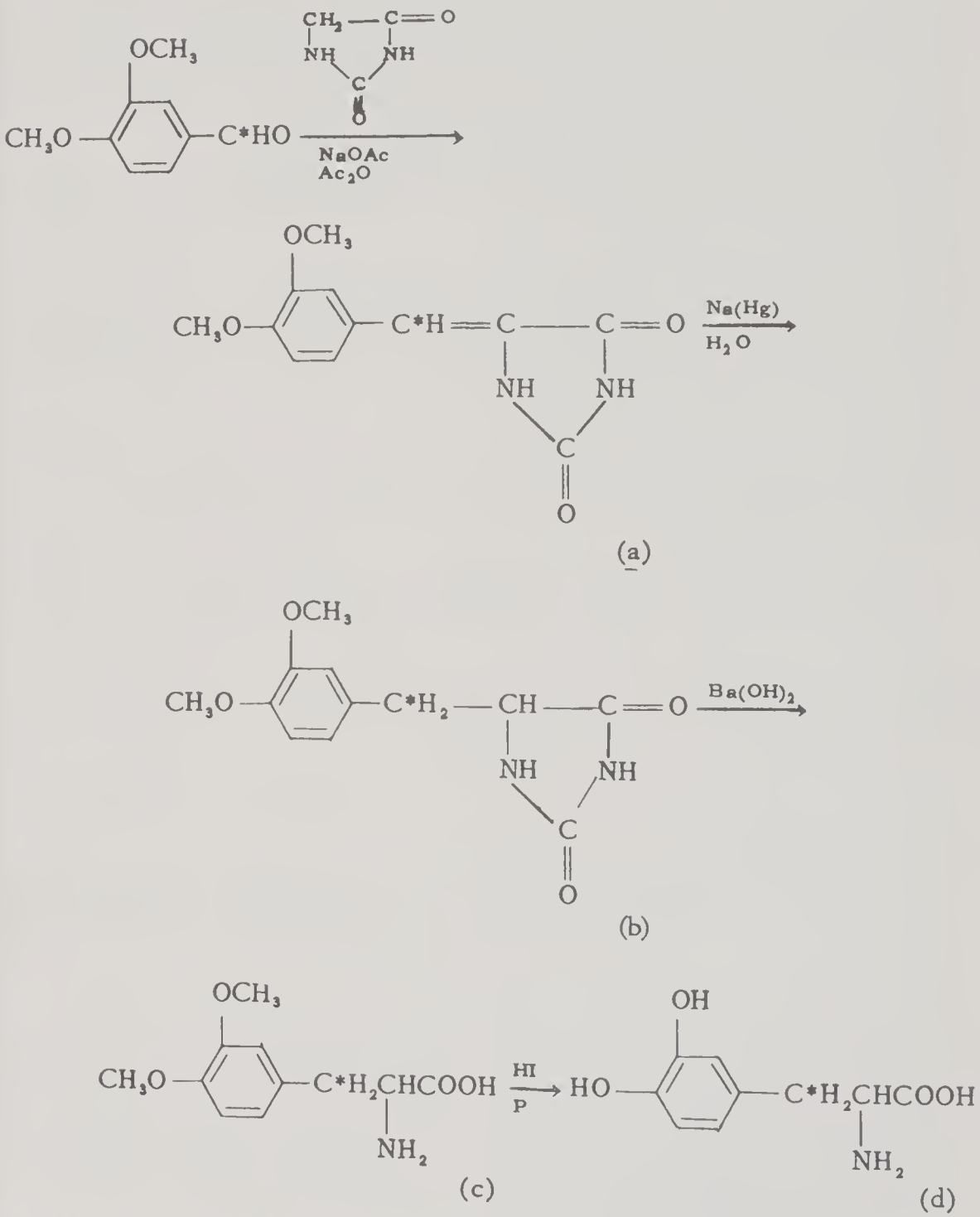
(b) *3-(3,4-Dimethoxyphenyl)alanine-1-C*^{14}. The 5-(3,4-dimethoxybenzyl)hydantoin-4-C^{14} is refluxed for 8 hours with a mixture of 8 g. of recrystallized barium hydroxide and 10 ml. of water. The warm solution is neutralized with 6 *N* sulfuric acid (methyl red), then cooled and filtered, and the precipitate is washed with four 10-ml. portions of hot water. The solution is concentrated to 2 ml.; then 5 ml. of alcohol is added, and the mixture is kept at 0° for 12 hours. The product is filtered, washed with alcohol and dried; yield 0.160 g., m.p. 240–245°.

(c) *3-(3,4-Dihydroxyphenyl)alanine-1-C*^{14}. A mixture of 0.160 g. of 3-(3,4-dimethoxyphenyl)alanine-1-C^{14}, 0.060 g. of 3-(3,4-dihydroxyphenyl)alanine, 2.5 ml. of redistilled hydriodic acid and 0.010 g. of red phosphorus is heated for 1 hour at 110° in a sealed, evacuated tube. The mixture is filtered, and the solution is evaporated to dryness under reduced pressure (nitrogen). The residue is dissolved in 3 ml. of water, the pH is adjusted to 6 with ammonia, and the mixture is evaporated to dryness. The residue is dissolved in 3 ml. of water and diluted with 20 ml. of alcohol. The solution is concentrated under reduced pressure (atmosphere of nitrogen) until precipitation occurs, then cooled to 0° for 1 hour and filtered. The mother liquor is treated similarly until no further product separates. The product (0.124 g.) with 0.080 g. of added carrier is recrystallized from 3 ml. of water saturated with sulfur dioxide (Norit); yield 0.103 g., m.p. 286–288°. The radiochemical yield is 8.5%.

B. Notes

1. Directions are given by Clemo for preparing the aldehyde bisulfite compound from ethyl chloroacetate and veratraldehyde by the Darzens synthesis. See 5-(*p*-methoxybenzyl)hydantoin-4-C^{14}.

3-(3,4-DIHYDROXYPHENYL)ALANINE-3-C^{14}



M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert, P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 225.

Procedure

(a) *5-Veratrylidene-C^{14}-hydantoin.* A mixture of 0.69 g. (4.1 mmoles) of crude veratraldehyde-C^{14}, 0.60 g. (6.0 mmoles) of hydantoin, 0.60 g. of

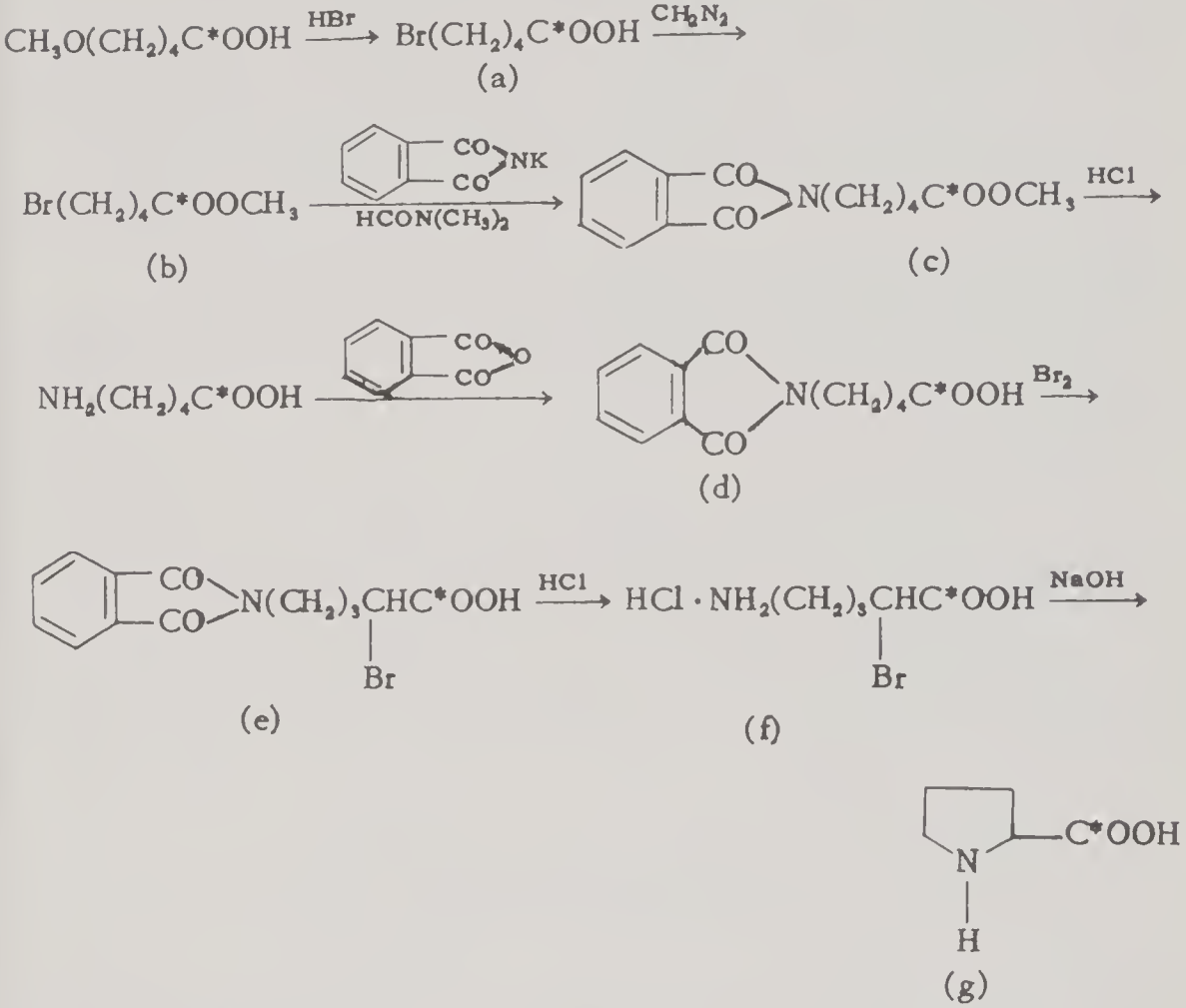
fused sodium acetate and 2.5 ml. of acetic anhydride is heated at 130° for 80 minutes in an evacuated, sealed, heavy-walled Pyrex tube. The reaction mixture is stirred for 5 minutes with 10 ml. of hot water, then diluted with hot water (30 ml.) until no further precipitate forms. The crude product is collected on a filter, without delay, washed with hot water and dried; yield 0.85 g. (83%), m.p. 176–186°. See the similar preparation of 5-(*p*-methoxybenzylidene-C^{14})hydantoin.

(b) *5-Veratryl-C^{14}-hydantoin.* To a stirred solution of the crude 5-veratrylidene-C^{14}-hydantoin in 25 ml. of 0.25 *N* sodium hydroxide is added 12 g. of 3% sodium amalgam over a period of 4 hours. After being stirred for an additional 7 hours, the solution is adjusted to pH 8 with concentrated hydrochloric acid, then treated with 2 g. of amalgam and stirred for 1.5 hours. The solution is separated by decantation, neutralized with hydrochloric acid, decolorized with carbon and concentrated to 5 ml. under reduced pressure. The product is collected, washed with three 2-ml. portions of cold water and dried; yield 0.50 g. (60%), m.p. 148–153°.

(c) *3-(3,4-Dimethoxyphenyl)alanine-3-C^{14}.* A mixture of the 5-veratryl-C^{14}-hydantoin, 10 g. of barium hydroxide monohydrate and 20 ml. of water is refluxed for 9 hours, then diluted with 10 ml. of water and treated (hot) with just enough 6 *N* sulfuric acid to precipitate the barium. The precipitate is filtered off and washed with four 20-ml. portions of hot water. The combined filtrate is concentrated to 2 ml. in an air stream, then diluted with 5 ml. of alcohol and stored in a refrigerator overnight. The product is collected, washed with two 3-ml. portions of alcohol and dried; yield 0.33 g. (73%), m.p. 215° (dec).

(d) *3-(3,4-Dihydroxyphenyl)alanine-3-C^{14}.* A mixture of the 3-(3,4-dimethoxyphenyl)alanine-3-C^{14}, 0.02 g. of red phosphorus and 4.5 ml. of distilled hydriodic acid (d. 1.7) is heated for 1 hour at 120° in an evacuated, sealed, heavy-walled Pyrex tube. The solution is filtered and evaporated under vacuum, the residual acid being removed by evaporation of 3 ml. of added water. A solution of the residue in 4 ml. of water is adjusted to pH 6 with concentrated ammonia and evaporated to dryness. The residue is washed with 3 portions of water (total 4 ml.) and two 1-ml. portions of alcohol, then dried in a vacuum desiccator; yield 0.23 g. (79%), m.p. 270–273° (dec.).

2-PYRROLIDINECARBOXYLIC-C^{14} ACID
(C_1^{14}-Proline)



L. Pichat, C. Baret, M. Audinot, M. Herbert and J. Lambin, *Radioisotope Conference, 1954*, Vol. I, Academic Press, New York, 1954, p. 245.

A. Procedure (Note 1)

(a) *5-Bromovaleric-1-C^{14} Acid.* Anhydrous hydrogen bromide is passed through 5-methoxyvaleric-1-C^{14} acid for 2 hours at room temperature, and 3 hours at 50°; then the excess is removed under vacuum at room temperature. The residue is dissolved in hot petroleum ether, and the solution is evaporated; yield 80–83% based on barium carbonate.

(b) *Methyl 5-Bromovalerate-1-C^{14}.* The acid is esterified quantitatively by the action of diazomethane in ether (Note 2).

(c) *Methyl 1,3-Dioxo-2-isoindolinevalerate-C^{14}, (Methyl 5-Phthalimidovalerate-1-C^{14})* (Note 3). A stirred mixture of 8.5 g. of potassium phthalimide, 40 mmoles of methyl 5-bromovalerate-1-C^{14} and 100 ml. of *N,N*-dimethylformamide is heated on a steam-bath for 2 hours. The mixture is cooled, and the potassium bromide is removed on a funnel and washed with the solvent (Note 4). The filtrate is evaporated under vacuum.

(d) *1,3-Dioxo-2-isoindolinevaleric-C^{14} Acid, (5-Phthalimidovaleric-1-C^{14} Acid).* A mixture of 5.0 mmoles of the ester and 30 ml. of concentrated hydrochloric acid is refluxed for 24 hours, cooled, diluted with 25 ml. of water and stored at 0°. The precipitated phthalic acid is filtered off, and the solution is evaporated under vacuum (Note 5). An aqueous solution of the residue is neutralized and again evaporated. The residual crude 5-aminovaleric-1-C^{14} acid is treated with 0.970 g. of phthalic anhydride, and the mixture is heated at 190° for 15 minutes, cooled, and crystallized from a hot solution of 12 ml. of alcohol and 68 ml. of water; yield 0.775 g. (63% based on 5-bromovaleric acid), m.p. 118–120°

(e) *α-Bromo-1,3-dioxo-2-isoindolinevaleric-C^{14} Acid, (2-Bromo-5-phthalimidovaleric-1-C^{14} Acid).* The acid is brominated by the method of Gaudry,[1] which follows.

A ground mixture of 12.35 g. (0.05 mole) of 5-phthalimidovaleric acid and 1.0 g. of red phosphorus is transferred to a flask containing 40 ml. of carbon tetrachloride. To the cooled, stirred mixture is added dropwise 10 ml. (0.20 mole) of bromine (Note 6). The mixture is refluxed for 4 hours and evaporated under reduced pressure. The residue is treated with 150 g. of crushed ice and stored in the cold for 24 hours to crystallize the oil. The product is collected and recrystallized from benzene; yield 15.97 g. (91.1%), m.p. 123–125°.

(f) *5-Amino-2-bromovaleric-1-C^{14} Acid Hydrochloride.* The phthalimido-acid is hydrolyzed by the method of Gaudry,[1] which follows.

A solution of 16.3 g. (0.05 mole) of 2-bromo-5-phthalimidovaleric acid and 250 ml. of concentrated hydrochloric acid is refluxed for 16 hours. The mixture is diluted with 250 ml. of water and cooled to crystallize the phthalic acid, which is filtered off, the last traces being removed by washing with two portions of ether. The acid solution is evaporated under reduced pressure (Note 7).

(g) *2-Pyrrolidinecarboxylic-C^{14} Acid.* The ring closure is carried out by the procedure of Gaudry,[1] which follows. Sodium chloride and bromide are removed by electrolysis in an apparatus with a circulating mercury cathode.[2] The over-all yield is 39% based on 5-bromovaleric acid at the 5-mmole level. Paper chromatography shows that the product is pure.

An aqueous solution of the 5-amino-2-bromovaleric acid hydrochloride and 20.0 g. of sodium hydroxide is stored at room temperature for 4 hours. The mixture is acidified with concentrated hydrochloric acid and evaporated under vacuum. The residue is dissolved in hot alcohol, the bulk of the sodium chloride is filtered off, and the filtrate is evaporated (Note 8).

B. Notes

1. The procedure is an adaptation of a method of Gaudry,[1] selected because of the intermediates involved, rather than because of its efficiency. A more direct route would have been from sodium cyanide-C^{14} and 4-hydroxybutyraldehyde (over-all yield 45%).[3]

2. Esterification through the acyl chloride is less satisfactory.

3. The procedure is an adaptation of the method of Sheehan.[4]

4. The yield of potassium bromide is 95.5%.

5. Selective partial hydrolysis, by different agents, gives poor yields, and the purity is not satisfactory.

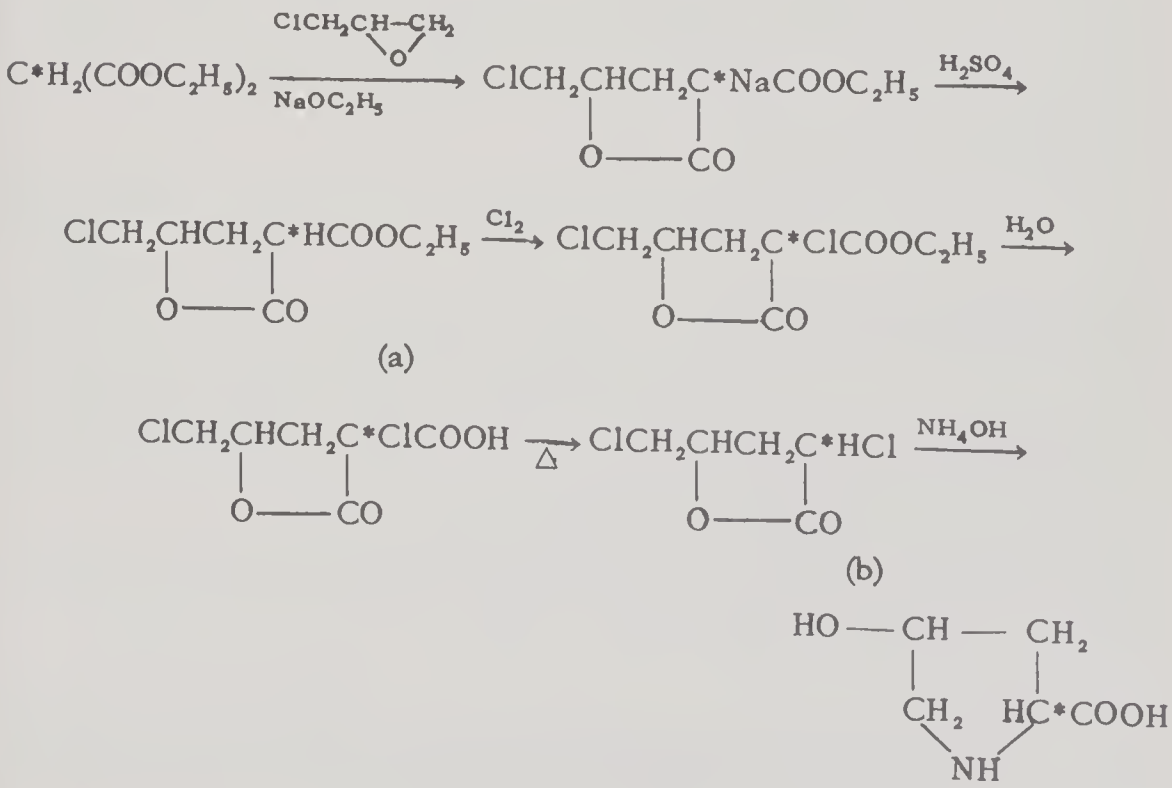
6. There is a vigorous evolution of hydrogen bromide.

7. The hydrochloride appears to be recrystallized with difficulty from alcohol-ether.

8. Purification of proline may be carried out through the copper salt,[3,5] which is formed by boiling an aqueous solution of the residue with excess copper carbonate for 1 hour, then concentrating the filtered solution and cooling; yield 4.85 g. (67.0%). A resolution is described by Velluz,[6] and a sensitive reaction on paper of proline with ninhydrin is described by Clarkson.[7]

[1]R. Gaudry and L. Berlinguet, Can. J. Research, *B28*, 245 (1950).
[2]R. Consden, A. H. Gordon and A. J. P. Martin, Biochem. J., *41*, 590 (1947);
[3]R. Gaudry, Can. J. Chem., *29*, 544 (1951). H. Plieninger, Ber., *83*, 271 (1950).
[4]J. C. Sheehan and W. A. Bolhofer, J. Am. Chem. Soc., *72*, 2786 (1950).
[5]R. Gaudry and L. Berlinguet, Can. J. Research, *27B*, 282 (1949).
[6]L. Velluz, G. Amiard and R. Heymes, Bull. soc. chim. France, (5) *21*, 1015 (1954).
[7]T. W. Clarkson, Biochem. Biophys. Acta, *18*, 453 (1955).

4-HYDROXYPROLINE-2-C^{14}



R. Gianetto and L. P. Bouthillier, Can. J. Biochem. and Physiol., *32*, 154 (1954).

A. Procedure

(a) *2-Carbethoxy-5-chloro-γ-valerolactone-2-C*^{14}. The sodium derivative of ethyl malonate-2-C^{14} is reacted with epichlorohydrin[1] according to the method of Traube,[2] which follows; yield 66%, b.p. 174–176° (cor.) (7–8 mm.).

To a solution of 19 g. (0.65 mole) of sodium in absolute ethanol is added 100 g. (0.625 mole) of ethyl malonate. The mixture is cooled, and to the suspension of ethyl sodiummalonate is slowly added 58 g. of epichlorohydrin in about three volumes of alcohol, while keeping the temperature below 50°. When the ethyl sodiummalonate is dissolved, the mixture is evaporated to dryness, and the residue is treated with dilute sulfuric acid. The freed lactone is extracted into ether, dried and collected by distillation, b.p. 180–182° (12 mm.); yield 65 g. (50%), sp. gr. 1.274 at 15°.

(b) *2,5-Dichloro-γ-valerolactone-2-C*^{14}. The procedure is an adaptation of the method of Leuchs,[3,2] which follows. The two racemic compounds formed in the chlorination step are not separated; yield 75%, b.p. 149–152° (cor.) (8–9 mm.).

The introduction of chlorine is carried out until the theoretical gain in weight is realized. After an initial latent period, there is a vigorous evolution of hydrogen chloride. Dry air is passed through the warmed mixture to remove dissolved gases. Upon long standing in a little absolute alcohol at refrigerator temperature, needles crystallize out, m.p. 55°. The filtered solution is evaporated under vacuum and the residue is distilled, b.p. 178–179° (10 mm.). Upon further long standing, a little more of the first isomer crystallizes from the oil, raising the yield of that isomer to 35%.

2-Carbethoxy-2,5-dichloro-γ-valerolactone (20 g.) is heated with 400 ml. of concentrated hydrochloric acid on a water-bath with frequent shaking until it goes into solution (1.5 hours). The mixture is evaporated under reduced pressure, and the residue is extracted into ether solution, which is washed with a little water to remove hydrochloric acid, then dried over sodium sulfate and evaporated. The 2-carboxy-2,5-dichloro-γ-valerolactone is decarboxylated by heating under vacuum at 140°, and the lactone I is collected by distillation; yield 9.3 g. (66%), b.p. 159–161° (cor.) (13 mm.), sp. gr. 1.422 at 19°.

The lactone II, prepared from the oily isomeric ester, is obtained in the same yield and with the same boiling point, but different specific gravity (1.437).

(c) *4-Hydroxyproline-2-C*^{14}, *(4-Hydroxy-2-pyrrolidinecarboxylic-2-C*^{14} *Acid)*. 2,5-Dichloro-γ-valerolactone-2-C^{14} is reacted with ammonium hydroxide according to the method of Leuchs,[3,4] which follows. The

product is separated from the 4-allohydroxyproline-2-C^{14} by fractional crystallization of the copper salts, followed by treatment with hydrogen sulfide. Purification is accomplished by crystallization from water-alcohol; yield 3% based on barium carbonate-C^{14}.

A mixture of 10 g. of 2,5-dichloro-γ-valerolactone I and 100 ml. of 25% ammonium hydroxide solution (and a little alcohol) is shaken for 2 days in a stoppered flask at room temperature. The mixture is evaporated on a water-bath, 23 g. of barium hydroxide is added to the residue, and heating is continued to decompose the ammonium salts and remove ammonia and water. The residue is dissolved in 200 ml. of hot water and treated with sufficient dilute sulfuric acid to precipitate the barium quantitatively. The cooled filtrate is treated similarly with silver carbonate to remove halide, then is heated with copper oxide for 1 hour to form a deep blue solution of the amino acid copper salts. The solution is concentrated to 200 ml. under vacuum and cooled to 0°, yielding 4.0 g. of a sky-blue salt (I). Concentration of the filtrate on a steam-bath yields 1.9 g. of a violet salt (II). The mother liquor is diluted with methanol, precipitating a greenish salt; the deep-blue filtrate is concentrated, as before, to yield 0.85 g. of the blue salt and 0.15 g. of the violet salt. Recrystallization of these latter fractions from 20 parts of hot water yields a total of 4.45 g. of the blue salt (38%) and 2.45 g. of the violet salt (26%). The salts are those of the I and II forms of 4-hydroxyproline, respectively, the former containing 4 molecules of water of crystallization which are lost by heating at 105°. Form II is 4-allohydroxyproline.[5]

The free acid is prepared by treating the copper salt in very dilute hydrochloric acid solution with hydrogen sulfide. After boiling off the excess reagent and treating the filtered solution with silver carbonate, the filtrate is concentrated to crystallize the acid. Purification is achieved by dissolution in a minimal volume of water and dilution with alcohol.

4-Hydroxyproline I melts at 255° (dec.), an aqueous solution tests neutral to litmus, and there is formed a 2-naphthalenesulfo-derivative,[6] m.p. 183–184°, which is fairly soluble in hot water.

4-Hydroxyproline II melts at 245° (dec.), an aqueous solution tests acidic to litmus, and there is formed a bis-2-naphthalenesulfo-derivative,[6] m.p. 178–179°, which is scarcely soluble in hot water.

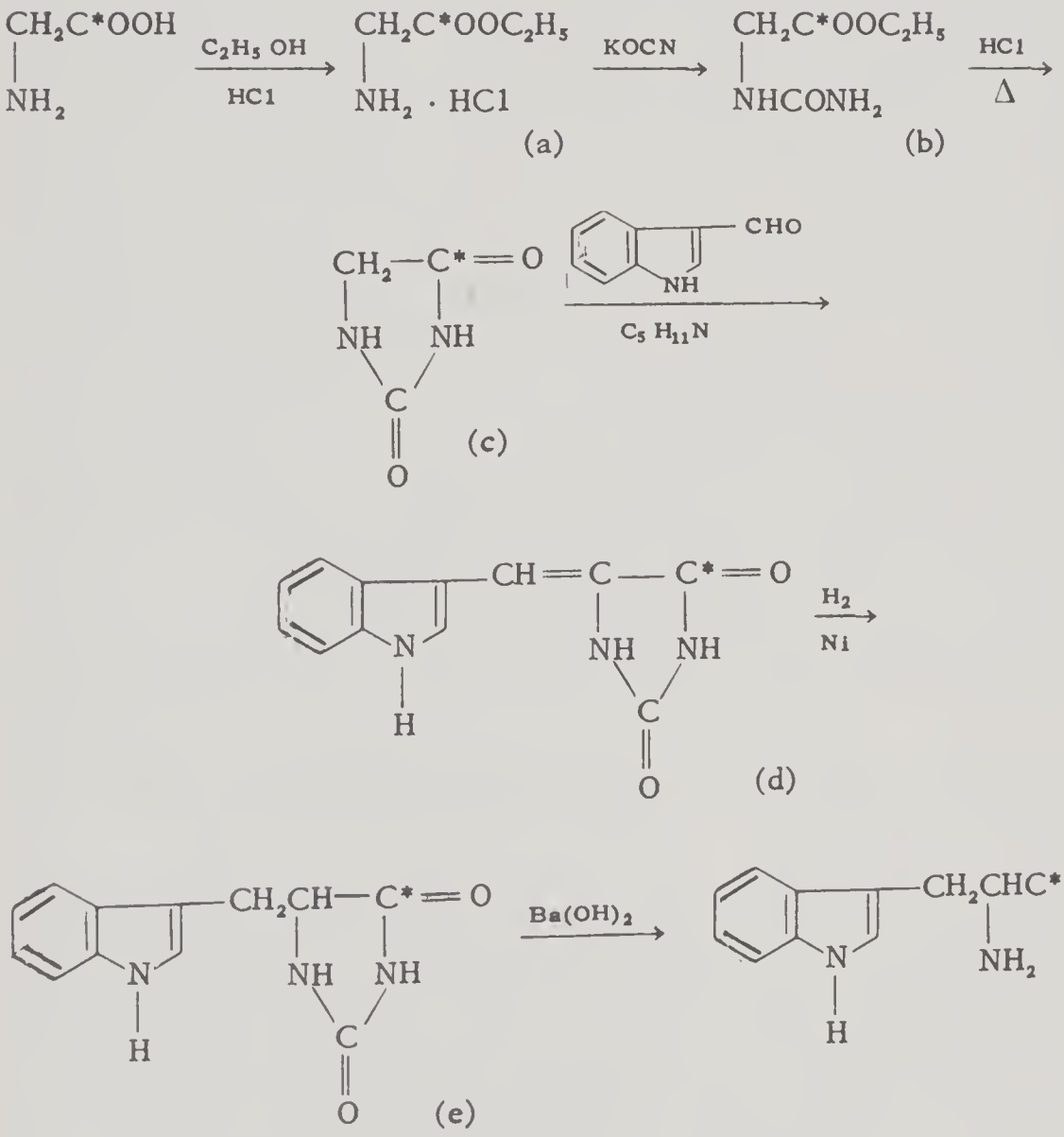
Treatment of 2,5-dichloro-γ-valerolactone II in the same manner yields the copper salts of 4-hydroxyproline: form I 29%, form II 20%.

A sensitive reaction on paper of hydroxyproline with ninhydrin is described by Clarkson.[7]

[1] *Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 233.
[2] W. Traube and E. Lehman, Ber., *34*, 1971 (1901); *32*, 720 (1899).

[3]H. Leuchs, M. Giua and J. F. Brewster, Ber., *45*, 1960 (1912).
[4]H. Leuchs, Ber., *38*, 1937 (1905).
[5]A. Neuberger, J. Chem. Soc., *1945*, 429.
[6]E. Fischer and P. Bergell, Ber., *35*, 3779 (1902).
[7]T. W. Clarkson, Biochem. Biophys. Acta, *18*, 453 (1955).

α-AMINO-3-INDOLEPROPIONIC-C^{13} ACID
(C_1^{13}-Tryptophan)



H. W. Bond, J. Biol. Chem., *175*, 531 (1948).

A. Procedure

(a) *Glycine-1-C^{13} Ethyl Ester Hydrochloride* (Note 1). Anhydrous hydrogen chloride is bubbled through a mixture of 1.93 g. of glycine-1-C^{13}

and 10 ml. of absolute ethanol heated in a bath at 65–70°. Alcohol is added, as needed, and the reaction is continued for 15 minutes after the disappearance of solid; then the mixture is evaporated to dryness on a warm surface; yield 3.49 g. (98.0%), m.p. 145.2°.

(b) *Ethyl Hydantoate-1-C^{13}*, (*Glycoluric-1-C^{13} Acid*) (Note 1). To a stirred solution of the glycine-1-C^{13} ethyl ester hydrochloride in 4 ml. of water is added a slurry of 3.0 g. of freshly prepared potassium cyanate[1] in 7 ml. of water. As soon as precipitation starts, the mixture is cooled at –5° for 2 hours; then the product is filtered off and dried; yield 3.40 g. (92.4%, m.p. 129.3–129.8°).

(c) *Hydantoin-4-C^{13}* (Note 2). A mixture of the ethyl hydantoate-1-C^{13} and 15 ml. of 25% hydrochloric acid is evaporated to dryness overnight on a steam-bath. The yield of crude product is 2.67 g., m.p. 203–206° (Note 3).

(d) *5-(3-Indolylmethylene)hydantoin-4-C^{13}* (Note 4). A mixture of 3.38 g. of 3-indolecarboxaldehyde, the hydantoin-4-C^{13} and 0.9 ml. of piperidine is refluxed for 20 minutes in an oil-bath preheated to 150°. The yellow solid is cooled, suspended in 250 ml. of water, acidified to Congo red with acetic acid, filtered off, and washed with water; yield 5.00 g. (94.5%), m.p. 314–315° (Note 5).

(e) *5-(3-Indolylmethyl)hydantoin-4-C^{13}* (Note 6). The 5-(3-indolylmethylene)hydantoin-4-C^{13} is suspended in 50 ml. of *N* sodium hydroxide and hydrogenated over 5 g. Raney nickel at room temperature and atmospheric pressure. The solid dissolves, and hydrogenation is complete within 8 hours. The filtered solution is acidified with dilute hydrochloric acid, and the product is collected; yield 3.57 g. (71.0%), m.p. 218° (Note 7).

(f) *α-Amino-3-indolepropionic-C^{13} Acid.* The 5-(3-indolymethyl) hydantoin-4-C^{13} is hydrolyzed for 40 hours by a modification of the method of Elks,[2] which follows. The yield is 2.80 g. (88.0%), m.p. 268° (Note 8).

A mixture of 13.8 g. of 5-(3-indolylmethyl)hydantoin, 69 g. of crystalline barium hydroxide and 420 ml. of water is refluxed for 24 hours; then the condenser is removed to expel the last traces of ammonia. The mixture is diluted and saturated with carbon dioxide to remove barium, the last traces of which are precipitated by the addition of 2 *N* sulfuric acid (rhodizonic acid indicator). The filtered solution is evaporated to dryness on a steam-bath, and the residue is washed with alcohol and dried.

B. Notes

1. The procedure is according to Harries and Weiss.[3] For isolation of the free base, see 2-aminoethanol-1-$C^{13}_{1/1}$-2-$C^{14}_{1/1}$.

2. The procedure is according to the methods of Harries[3] and Wagner.[4]

3. The theoretical weight is 2.33 g., and pure hydantoin melts at 216°. Purification is unnecessary, but may be effected by alcohol extraction.[4] The yield approaches 100%.

4. The procedure is according to Shabica,[5] who also describes the preparation of the starting material, by formylating either indole or ethyl 2-indolecarboxylate with *N*-methylformanilide.

5. An authentic sample melts at 314° (metal block).

6. The procedure is that of Elks.[2]

7. If the catalyst is not sufficiently active, the hydrogenation time is prolonged beyond the usual 6 hours, and partial hydolysis results in a product melting over a wide range. The yield is usually about 90%.

8. The product shows full biological activity. The yield is 59% from hydantoin.

C. Other Preparations

The preparation of glycine-1-C^{13}-2-C^{14} ethyl ester hydrochloride by the described method has been reported by Weissbach;[6] yield 57% based on sodium acetate, m.p. 142–143°.

Glycine-2-C^{14} ethyl ester hydrochloride has been prepared[7] in 94.4% yield at the 27-mmole level by esterification of glycine-2-C^{14} essentially as described.

[1] *Inorganic Syntheses*, Vol. 2, McGraw, New York, 1946, p. 87.

[2] J. Elks, D. F. Elliott and B. A. Hems, J. Chem. Soc., *1944*, 629.

[3] C. D. Harries and M. Weiss, Ber., *33*, 3418 (1900).

[4] C. E. Wagner and J. K. Simons, J. Chem. Educ., *13*, 265 (1936).

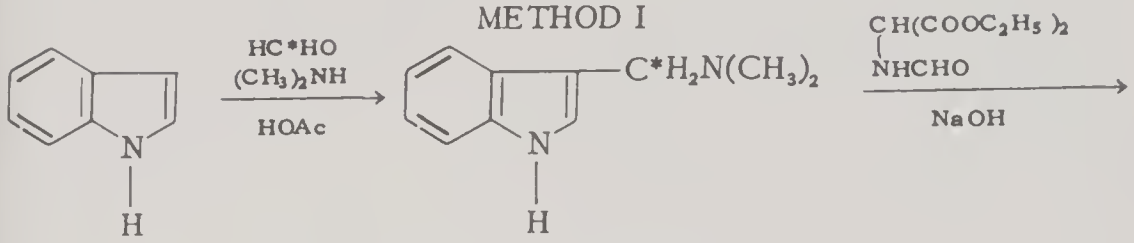
[5] A. C. Shabica, E. E. Howe, J. B. Ziegler and M. Tishler, J. Am. Chem. Soc., 68, 1156 (1946); W. J. Boyd and W. Robson, Biochem. J., *29*, 2256 (1935).

[6] A. Weissbach and D. B. Sprinson, J. Biol. Chem., *203*, 1031 (1953).

[7] F. Acree, Jr., C. C. Roan and F. H. Babers, J. Econ. Entomol., *47*, 1066 (1954).

TRYPTOPHAN-β-C^{14}
(α-Amino-3-indolepropionic-β-C^{14} Acid)

METHOD I



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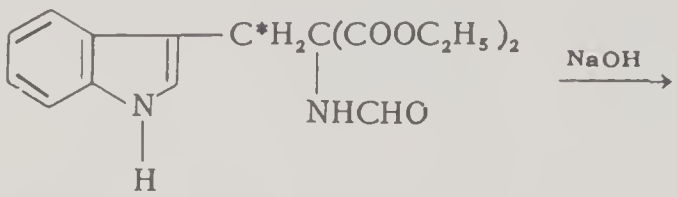

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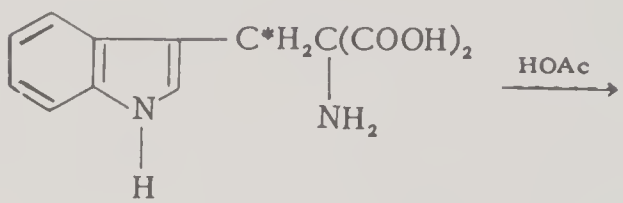
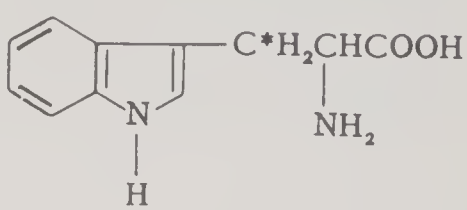




(c)

F. Weygand and H. Linden, Z. Naturforsch., *9b*, 682 (1954).

A. Procedure

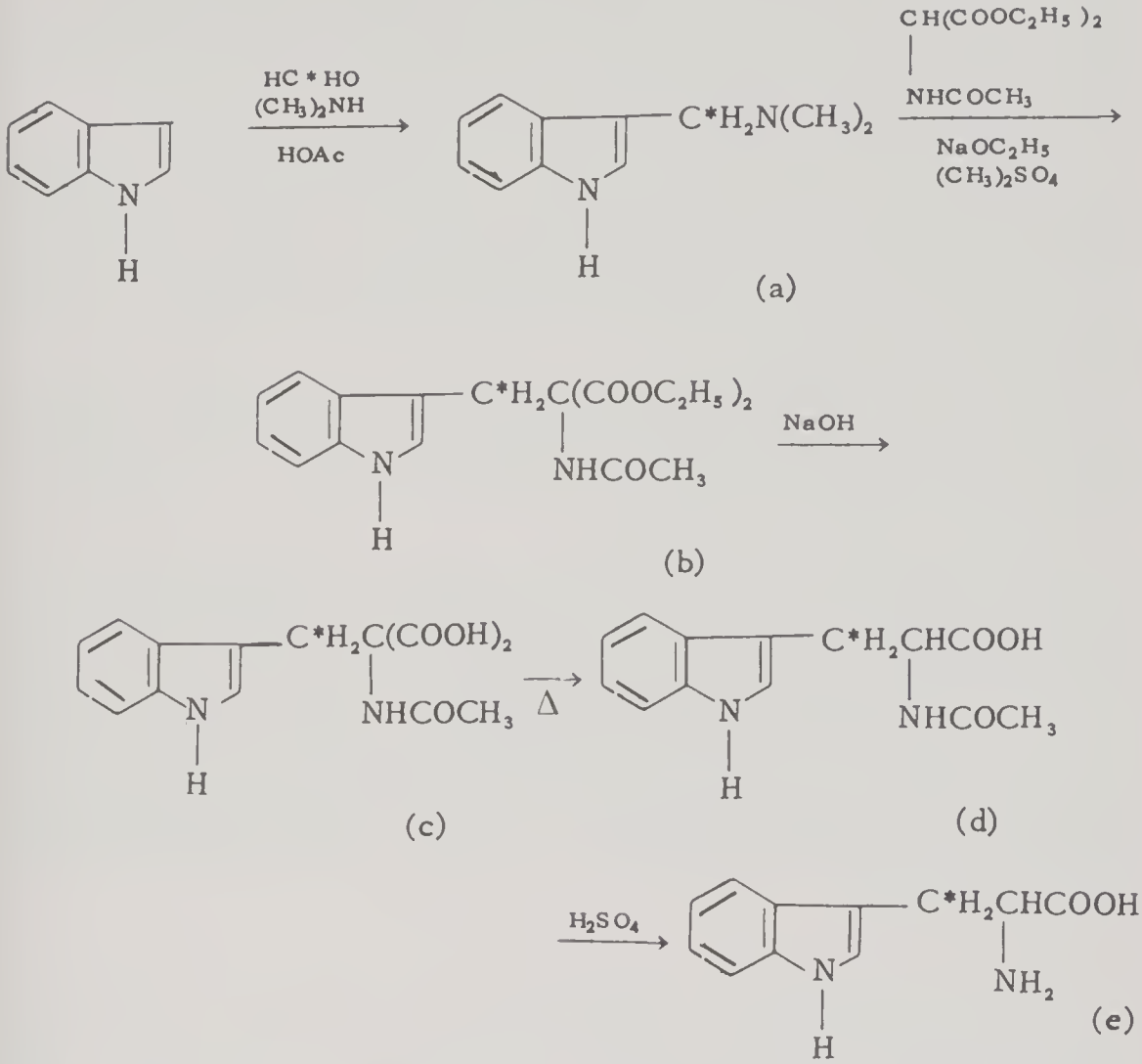
(a) *3-(Dimethylaminomethyl-C^{14})indole, (C_1^{14}-Gramine).* The dry formaldehyde-C^{14} complex prepared from 2.0 mmoles of barium carbonate-C^{14} (see formaldehyde-C^{14}, Method I) is decomposed at –80° by the addition of 1.0 ml. of methanol and is treated with an ice-cooled solution containing 0.428 g. (9.5 mmoles) of dimethylamine, 5 ml. of glacial acetic acid and 1 ml. of water. The closed flask is shaken for 1 minute; then the mixture is treated with 0.234 g. (2.0 mmoles) of indole and shaken occa-

sionally during 20 hours. The mixture is chilled, made alkaline with 4 g. of sodium hydroxide in 40 ml. of water and stored overnight in a refrigerator. The precipitate is collected, washed with three 10-15-ml. portions of water (Note 1) and extracted with 40 ml. of tetrahydrofuran. The solvent is evaporated, and the brown residue is dissolved in 50 ml. of hot petroleum ether (60-90°) (Note 2). The solution is concentrated to 30 ml. and stored overnight in a refrigerator; yield 0.117 g. An additional crop of product is obtained by concentrating the mother liquor to 3 ml., making the total yield 0.155 g. (46% based on barium carbonate), m.p. 124-126°.

(b) *Ethyl Formamido(3-indolylmethyl-C^{14})malonate* (Note 3). A mixture of 0.155 g. of the gramine, 0.272 g. of ethyl formamidomalonate,[1] about 0.010 g. of powdered sodium hydroxide and 2.5 ml. of absolute xylene is refluxed for 7 hours in a stream of nitrogen. The mixture is stored overnight in a refrigerator, and the product is collected and washed with petroleum ether; yield 0.284 g. (97.5%), m.p. 167-169°. Purification is accomplished by recrystallization from 2 ml. of xylene, washing with petroleum ether and drying under vacuum at 60° over paraffin; yield 0.264 g. (90.5%), m.p. 177°.

(c) *Tryptophan-β-C^{14}*. A mixture of 0.256 g. of the malonic ester and 1.6 ml. of sodium hydroxide solution (2.3 g./22 ml.) is heated at 120-130° under reflux for 6 hours, then treated with 0.3 ml. of glacial acetic acid, and the heating is continued for 2 hours. The mixture is stored in refrigerator, and the product is collected, washed with a little icewater and recrystallized from acetic acid-benzene; yield 0.119 g., m.p. 235° (dec.). Further purification is accomplished by recrystallization from boiling water-alcohol. The yield is 69% based on gramine.

METHOD II



C. Heidelberger, J. Biol. Chem., *179*, 139 (1949).

A. Procedure

(a) 3-(*Dimethylaminomethyl-C*^{14})*indole* (Note 4). Aqueous formaldehyde-C^{14} (10–15 mmoles, Note 5) is added to a chilled mixture of 1.42 g. of 33% aqueous dimethylamine and 1.42 g. of glacial acetic acid; then the solution is added quickly to 1.10 g. indole. The solution is stored at room temperature for 18 hours, then added dropwise to a stirred, ice-cold solution of 1.42 g. of sodium hydroxide in 20 ml. of water. The crude crystalline product is collected, washed with a little cold water and dried; yield 1.39 g. (33% based on barium carbonate), m.p. 121–126° (Note 6). Degradation studies indicate that there is no isotopic rearrangement (Note 7).

(b) *Ethyl Acetamido(3-indolylmethyl-C*^{14})*malonate* (Note 8). To a stirred solution of 0.18 g. of sodium is about 15 ml. of absolute ethanol is added

1.70 g. of ethyl acetamidomalonate,[2] followed by 1.29 g. of crude 3-(dimethylaminomethyl-C^{14})indole; methyl sulfate (1.2 ml.) is added slowly with stirring (Note 9), and the mixture is stored at room temperature for 4 hours, then poured into ice-water and chilled to crystallize the crude product; yield 2.10 g. (82%), m.p. 131–133.5°.

(c) *Acetamido(3-indolylmethyl-C^{14})malonic Acid* (Note 10). A mixture of 2.10 g. of the ester, 1.20 g. of sodium hydroxide and 10 ml. of water is refluxed for 3 hours. The mixture is cooled and acidified with concentrated hydrochloric acid to precipitate the product, which is collected and dried in a desiccator over calcium chloride; yield 1.57 g. (90%), m.p. 135–138° (dec.) (Note 11).

(d) *α-Acetamido-3-indolepropionic-β-C^{14} Acid.* The decarboxylation of the malonic acid is effected smoothly by heating to 140°. The product is recrystallized from alcohol-water containing a trace of sodium hydrosulfite; yield 92%, m.p. 206–208°.

(e) *Tryptophan-β-C^{14}.* A mixture of 0.53 g. of α-acetamido-3-indolepropionic-β-C^{14} acid and 6 ml. of 2 *N* sulfuric acid is refluxed for 4 hours under nitrogen. The mixture is diluted with 30 ml. of water, decolorized with charcoal and neutralized to phenolphthalein with barium hydroxide. The hot solution is filtered and evaporated to dryness; then the crude tryptophan-β-C^{14} is recrystallized (almost quantitatively) from 15 ml. of acetic acid and 15 ml. of benzene. The yield is 0.54 g. (82%) of tryptophan-β-C^{14} acetate containing one molecule of acetic acid of crystallization (Note 12).

B. Notes

1. The filtrate may be saved for recovery of methanol-C^{14} and sodium formate-C^{14}.

2. An insoluble brown smear of 3,3′-methylene-C^{14}-diindole remains.

3. The procedure is an adaptation of that of Hellmann.[1]

4. The method is adapted from that of Snyder.[3] The isotopic preparation has been made also by Bowden[4] and by Stutz[5] (75–80% yield based on barium carbonate, employing vacuum line technique).

5. The formaldehyde-C^{14} was prepared in 40–60% yield by the catalytic oxidation of methanol-C^{14} over copper, following the reduction of 25 mmoles of carbon-C^{14} dioxide to the alcohol.

6. Albertson[6] has reported yields of 90–93% for this step. Stutz[5] has described the preparation in 92–97% yield (m.p. 131–133°) based on formaldehyde through the use of vacuum line technique.

7. The degradation was performed by Bowden[4] and Leete.[7] 3-(Dimethylaminomethyl-C^{14})indole is reacted with ethyl iodide in the presence of potassium ethoxide, yielding inactive *N,N*-dimethylethylamine (picrate), and 3-(ethoxymethyl)indole having the same molar specific activity as the

starting material. Fusion of 3-(dimethylaminomethyl-C^{14})indole with potassium hydroxide yields potassium 3-indolecarboxylate-C^{14}, which is converted to 3-indolecarboxylic-C^{14} acid and the *S*-benzylisothiuronium salt for assay. Both derivatives have the same molar specific activity as the starting gramine. The free acid is decarboxylated by heating, forming nonisotopic indole and carbon-C^{14} dioxide containing all of the original activity.

8. The procedure is a suitable modification of those of Albertson[6] and Snyder.[2] The isotopic preparation has been made also by Bowden.[4]

9. The reaction is exothermic. An alternate procedure, described by Albertson,[6] employs methyl iodide and apparently gives the same yield of product, but with a greater recovery of gramine. The starting material is recovered from the filtrate, after removal of alcohol, by extracting the aqueous solution with chloroform, then washing the extract with acid and basifying the acid solution.

10. The procedure is adapted from the method of Snyder and Smith.[2]

11. The product may be purified by recrystallization from 10 volumes of water at 50° containing a trace of sodium hydrosulfite. The melting point is 144.5°, after two recrystallizations from 20% ethanol.

12. A degradation of C^{14}-tryptophan, which allows the isolation of each carbon atom as carbon dioxide, has been described by Rafelson.[8] Some of the degradation products might be of value as synthetic intermediates.

C. Other Preparations

In a similar manner, starting with ethyl acetamidocyanoacetate-2-C^{14} and gramine, is prepared[9] ethyl α-acetamido-α-cyano-3-indolepropionate-α-C^{14}. Hydrolysis by refluxing for 5 hours in sodium hydroxide solution, followed by acidification with acetic acid and storage at 0°, yields tryptophan-α-C^{14} (69% yield for the hydrolysis).

In a similar manner, starting with indole-2-C^{14}, is prepared[7] 3-(dimethylaminomethyl)indole-2-C^{14}, ethyl acetamido(3-indolyl-2-C^{14}-methyl)-malonate, α-acetamido-3-indolepropionic-2-C^{14} acid and tryptophan-2-C^{14}.

The preparation of 3-(dimethylaminomethyl)indole-2-C^{14}, from indole-2-C^{14} by a similar Mannich reaction, has been described by Pichat;[10] yield 90–93%, m.p. 132°.

From indole-3-C^{14} by Method II has been prepared[11,12] tryptophan-3-C^{14} by the intermediate sequence: 3-(dimethylaminomethyl)indole-3-C^{14}, ethyl acetamido(3-indolylmethyl-3-C^{14})malonate, acetamido(3-indolylmethyl-3-C^{14})malonic acid and α-acetamido-3-indolepropionic-3-C^{14} acid. The overall radiochemical yield of tryptophan-3-C^{14} acetate (1 molecule of acetic acid of crystallization) was 6% based on pyruvamide-3-C^{14}.

[1]H. Hellmann, Hoppe-Seyler's Z. physiol. Chem., *284*, 163 (1949).
[2]H. R. Snyder and C. W. Smith, J. Am. Chem. Soc., *66*, 350 (1944).

[3]H. R. Snyder, C. W. Smith and J. M. Stewart, J. Am. Chem. Soc., *66*, 200 (1944).

[4]K. Bowden and L. Marion, Can. J. Chem., *29*, 1036 (1951).

[5]R. E. Stutz, D. E. Atkinson and S. A. Gordon, Atomic Energy Commission Report, ANL-4710; Nuc. Sci. Abstracts, *6*, 164 (1952).

[6]N. F. Albertson, S. Archer and C. M. Suter, J. Am. Chem. Soc., *67*, 36 (1935).

[7]E. Leete and L. Marion, Can. J. Chem., *31*, 1195 (1953).

[8]M. E. Rafelson, Jr., G. Ehrensvärd, M. Bashford, E. Saluste and C. G. Heden, J. Biol. Chem., *211*, 725 (1954); M. E. Rafelson, Jr., J. Biol. Chem., *212*, 953 (1955).

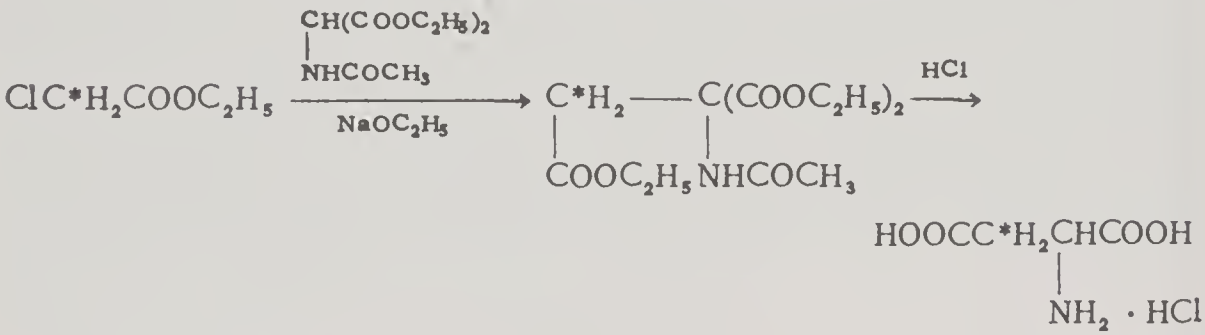
[9]M. Fields, Nucleonics, 7 (3), 64 (1950).

[10]L. Pichat, M. Audinot and J. Monnet, Bull. soc. chim. France, (5) 21, 85 (1954).

[11]E. Leete, L. Marion and I. D. Spenser, Can. J. Chem., *33*, 405 (1955).

[12]C. Heidelberger, J. Biol. Chem., *179*, 139 (1949).

ASPARTIC-3-C^{14} ACID HYDROCHLORIDE
(2-Aminosuccinic-3-C^{14} Acid Hydrochloride)



R. M. Noller and B. M. Tolbert, Atomic Energy Commission Report, UCRL-2041; Nuc. Sci. Abstracts, 7, 2533 (1953); Chem. Abstracts, 48, 12014 (1954).

A. Procedure (Note 1)

In a solution of 7.32 g. (33.8 mmoles) of ethyl acetamidomalonate in 40 ml. of absolute alcohol is dissolved 0.51 g. (22.2 mmoles) of sodium. To the solution is added, in 5–10 ml. of absolute alcohol, the crude ethyl chloroacetate-2-C^{14} prepared from 22.5 mmoles of sodium acetate-2-C^{14} (Note 2); then the mixture is refluxed on a steam-bath for 4 hours (Note 3). The solvent is distilled, the last traces being removed at 300 mm. The residue is refluxed with 50 ml. of concentrated hydrochloric acid for 72 hours; then the mixture is evaporated to dryness on the steam-bath and dried under vacuum. To an aqueous solution of the crude product is added a solution of 7.67 g. (33% molar excess) of hydrated cupric chloride (Note 4); the solution is diluted to 150 ml , then adjusted to pH 5 with *N* sodium hydroxide (97 ml.) and stored in a refrigerator for at least 2 days. The cold mixture is treated with 4 cc. of Celite, and the precipitate is collected by filtration on a bed of Celite, washing free of chloride with ice water. The precipitate is dispersed in 150 ml. of water containing 0.5

ml. of concentrated hydrochloric acid, and the stirred solution is saturated with hydrogen sulfide for 2.5 hours. The mixture is heated on the steam-bath for 15 minutes; then the solution is filtered through a bed of Celite (Note 5), treated with 25 ml. of centrated hydrochloric acid and evaporated to dryness. The residue is dissolved in 50 ml. of 6 *N* hydrochloric acid, again evaporated to dryness, then dissolved in a small amount of water and decolorized with carbon. The yield is 2.19 g., 66% based on sodium acetate-2-C^{14}; the molar specific activity agrees with that of the starting material. Two-dimensional paper chromatography shows that the only isotopic contaminant is less than 1% of a non-amino acid (Note 6).

B. Notes

1. The procedure is a modification of the method of Galat;[1] also see aspartic-4-C^{14} acid.

2. The yield of ester is 70–80%.

3. As sodium chloride quickly precipitates, stirring may be advisable to control bumping.

4. Purification is accomplished readily through the copper salt. Probably the most efficient method of purification is by the tedious procedure of elution from an ion exchange column.

5. Most of the activity is washed from the residue with dilute hydrochloric acid (1:200).

6. The impurity may be a chromatographic anomaly. The free acid may be precipitated from a concentrated aqueous solution of the hydrochloride by standing for 24 hours with the calculated amount of pyridine in 95% alcohol. The solubility of L-aspartic acid is 0.267 g. per 100 ml. of water at 0.2°. Aspartic acid melts with decomposition at 225–248° (m.p. 278–280° dec. in a sealed capillary with a preheated bath), the hydrochloride decomposes at 180–185°, and the *N*-benzoyl derivative melts at 160–162°.[1] Degradation methods are given by Ehrensvärd[2] and Mosbach.[3]

C. Other Preparations

Aspartic-4-C^{14} acid is prepared in the same manner from ethyl chloroacetate-1-C^{14}.

Aspartic-3-C^{13}-4-C^{14} acid has been prepared by Ehrensvärd[2] in a similar manner, starting with ethyl bromoacetate-2-C^{13}-1-C^{14}.

Aspartic-3-C^{14} acid has been prepared by Weinhouse[4] by essentially the the method described, using equimolar quantities. The substituted malonic ester was hydrolyzed and decarboxylated by heating with hydrobromic acid. The dried hydrobromide was treated with ammonium hydroxide and, after evaporation to dryness, the ammonium bromide was extracted with

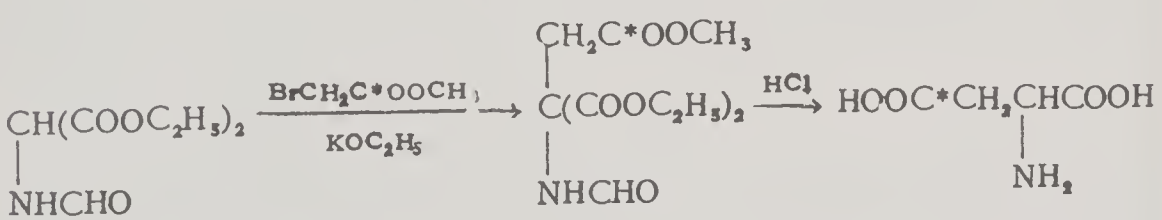
methanol. The residual product was recrystallized from water; yield 30% based on ethyl bromoacetate-2-C^{14}. Use of this method has been reported Delluva[5] and Weed.[6]

The preparation of aspartic-3-C^{14} acid by condensation of ethyl bromoacetate-2-C^{14} with ethyl formamidomalonate, after the method described for the synthesis of aspartic-4-C^{14} acid, has been described by Kögl;[7] yield 61.5% at the 24-mmole level.

Aspartic-3-C^{14} acid has been formed[8] by hydrogenation of 0.020 g. of 2-aminofumaramide-3-C^{14} over an excess of Adams catalyst for 2 hours in glacial acetic acid at atmospheric pressure, the resulting material then being hydrolyzed by boiling with acetic-hydrochloric acid and evaporated to dryness. Chromatography on Whatman No. 1 paper with pyridine-water (65:35) gave a single spot, R_f 0.41, in agreement with an authentic sample.

[1]A. Galat, J. Am. Chem. Soc., *69*, 965 (1947).
[2]G. Ehrensvärd, L. Reio, E. Saluste and R. Stjernholm, J. Biol. Chem., *189*, 93 (1951).
[3]E. H. Mosbach, E. F. Phares and S. F. Carson, Arch. Biochem. Biophys., *33*, 179 (1951).
[4]S. Weinhouse and D. Wilson, Nucleonics, 7 (3), 61 (1950).
[5]A. M. Delluva, Arch. Biochem., *45*, 443 (1953).
[6]L. L. Weed and D. W. Wilson, J. Biol. Chem., *207*, 439 (1954).
[7]F. Kögl, P. Emmelot and D. H. W. Den Boer, Ann., *589*, 1 (1954).
[8]C. Cooper, R. Wu and D. W. Wilson, J. Biol. Chem., *216*, 37 (1955).

ASPARTIC-4-C^{14} ACID



S. C. Wang, T. Winnick and J. P. Hummel, J. Am. Chem. Soc., *73*, 2390 (1951).

A. Procedure (Note 1)

To a solution prepared by adding 5.58 g. (27.5 mmoles) of ethyl formamidomalonate to a solution of 1.06 g. of potassium in 25 ml. of absolute ethanol (Note 2) is added 25 mmoles of methyl bromoacetate-1-C^{14} in 23 ml. of absolute ethanol. The mixture is stored overnight (Note 3), then refluxed for 2.5 hours and cooled, and the precipitated salt is filtered off. The filtrate is evaporated under reduced pressure, and the residue is refluxed for 3 hours with 37.5 ml. of concentrated hydrochloric acid. The solution is evaporated under vacuum, the residue is dissolved in 12 ml. of water, and the pH is adjusted to 3.0 with concentrated ammonia; then the mixture is stored in a refrigerator for 2 days to crystallize; yield 2.29

g., m.p. 320–325° (block) (dec.) (Note 4). After decolorization with charcoal and recrystallization from boiling water, the combined yield is 63% (Note 5).

B. Notes

1. The procedure is a modification of the method of Galat.[1]

2. The use of methoxide markedly lowers the yield. Potassium gives a slightly higher yield than does sodium. A 10% molar excexs of ethyl formamidomalonate is optimal.

3. The yield is not increased by extending the period of standing to 2 days.

4. The mother liquor deposits an additional crop of crystals upon standing for 1 week at 3°.

5. Trial runs with ethyl bromoacetate have given yields of 71–78%.

C. Other Preparations

Aspartic-4-C^{14} acid hydrochloride has been prepared by Noller and Tolbert (approximately 66% yield based on sodium acetate-1-C^{14}); see aspartic-3-C^{14} acid hydrochloride. The conversion of aspartic-4-C^{14} acid to *N*-carbamoylaspartic-4-C^{14} acid, m.p. 177–178°, has been reported by Werkheiser;[2] see *N*-carbamoyl-C^{14}-aspartic acid.

[1]A. Galat, J. Am. Chem. Soc., *69*, 965 (1947).

[2]W. C. Werkheiser and D. W. Visser, Cancer Research, *15*, 644 (1955); J. F. Nyc and H. K. Mitchell, J. Am. Chem. Soc., *69*, 1382 (1947).

L-ASPARTIC-2,3-$C^{14}_{1/2}$ ACID

$$BrC^*H_2COOC_2H_5 \xrightarrow[NaOC_2H_5]{CH_2(COOC_2H_5)_2} \underset{\displaystyle COOC_2H_5}{\overset{\displaystyle |}{C^*H_2CH(COOC_2H_5)_2}} \xrightarrow{Br_2}$$

(a)

$$\underset{\displaystyle COOC_2H_5}{\overset{\displaystyle |}{\underset{\displaystyle |}{C^*H_2C(COOC_2H_5)_2}}} \xrightarrow{HCl} \underset{\displaystyle C^*HCOOH}{\overset{\displaystyle ||}{CHCOOH}} \xrightarrow[Cat.]{NH_4Cl} \underset{\displaystyle COOH}{\overset{\displaystyle |}{\underset{\displaystyle |}{C^*H_2C^*HCOOH}}}$$

(b) Br (c) NH_2 (d)

A. San Pietro, J. Biol. Chem., *198*, 639 (1952).

A. Procedure

(a) *Ethyl 2-Carbethoxysuccinate-3-C^{14}, (Ethyl Carbethoxymethyl-C^{14}-malonate).* Ethyl bromoacetate-2-C^{14} (45.8 mmoles) is condensed with

ethyl sodiummalonate in absolute ethanol according to the method of Bischoff[1] and of Kay and Perkin,[2] which follows. The yield is 47%, b.p. 160–170° (15 mm.).

Ethyl malonate (160 g.) is added slowly with cooling to a stirred solution of 23 g. of sodium in 350 ml. of absolute alcohol, followed by the slow addition of 160 g. of ethyl bromoacetate. The crude product is extracted from aqueous solution into ether and dried over sodium sulfate, and the solvent is evaporated. Purification is effected by fractional distillation (Note 1).

(b) *Ethyl 2-Bromo-2-carbethoxysuccinate-3-C^{14}.* The tricarboxylic ester is brominated in carbon tetrachloride with 10% excess of bromine according to the procedure of Palmer and McWherter.[3] The yield on the molar scale is 73–75%.

(c) *Fumaric-2-C^{14} Acid.* The crude ethyl-2-bromo-2-carbethoxysuccinate-3-C^{14} is refluxed with 6 *N* hydrochloric acid, with loss of carbon dioxide, ethanol and hydrogen bromide according to the method of Bischoff.[4] The product is purified by vacuum sublimation; yield 41% based on ethyl 2-carbethoxysuccinate-3-C^{14}, m.p. 282° (block).

(d) *L-Aspartic-2,3-$C^{14}_{1/2}$ Acid* (Note 2). To an aqueous solution of 0.565 g. of fumaric-2-C^{14} acid, neutralized with sodium hydroxide and diluted to 22 ml., is added 1.19 g. of ammonium chloride, 17.7 ml. of Clark and Lubs 0.05 *M* phosphate buffer at pH 7.4, 5 ml. of E. *coli* suspension and 4 ml. of toluene (Note 3). The flask is evacuated and filled with nitrogen, and the mixture is stored at 37° for 2 days, at which time equilibrium is reached. The mixture is centrifuged, and the supernatant liquid is separated, concentrated to small volume on a steam-bath and filtered. To the filtrate is added about 3 ml. of hot saturated copper sulfate solution, and the mixture is stored overnight in a refrigerator (Note 4). The crystals of copper aspartate-2,3-$C^{14}_{1/2}$ are collected and washed with a little cold water, then suspended in hot water, and the mixture is saturated with hydrogen sulfide to decompose the salt. The product, crystallized from the concentrated filtrate by the addition of two volumes of alcohol and standing overnight, is collected and washed with alcohol and with ether; yield 47%.

B. Notes

1. The ethyl ethanetricarboxylate always is accompanied by some ethyl propanetetracarboxylate.

2. The procedure is adapted from the procedures of Wu and Rittenberg[5] and of Quastel and Woolf.[6]

3. The stock preparation of resting cells, made up according to the directions of Wu and Rittenberg,[5] may be kept for at least 2 months.[4] In the presence of a growth inhibitor, such as toluene, no reduction to suc-

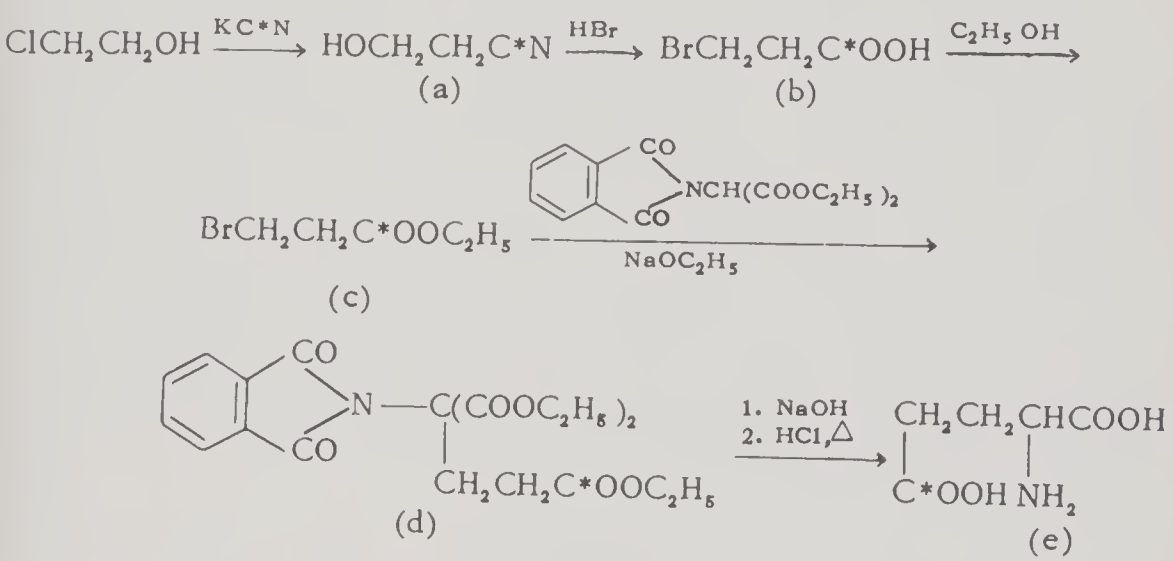
cinic acid occurs and the danger of exceeding the time necessary for the optimum yield of aspartic acid is eliminated.

4. Quastel and Woolf[6] recommend boiling a more dilute copper mixture for 15–30 minutes and filtering from a bulky precipitate, prior to crystallizing the salt. The yield of copper aspartate trihydrate is 3.5 g. from 3 g. of fumaric acid.

[1]C. A. Bischoff, Ann., *214*, 38 (1882).
[2]F. W. Kay and W. H. Perkin, Jr., J. Chem. Soc., *89*, 1640 (1906).
[3]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 245.
[4]C. A. Bischoff, Ber., *13*, 2161 (1880).
[5]H. Wu and D. Rittenberg, J. Biol. Chem., *179*, 847 (1949).
[6]J. H. Quastel and B. Woolf, Biochem. J., *20*, 545 (1926).

GLUTAMIC-5-C^{14} ACID
(2-Aminopentanedioic-5-C^{14} Acid)

METHOD I



R. J. Speer, A. Roberts, M. Maloney and H. R. Mahler, J. Am. Chem. Soc., *74*, 2444 (1952).

A. Procedure

(a) *Hydracrylonitrile-1-C^{14}, (Ethylene Cyanohydrin-C^{14})*. A mixture of 4 g. (50 mmoles) of ethylene chlorohydrin, 1.3 g. (20 mmoles) of potassium cyanide-C^{14}, 0.9 ml. of water, and 5 ml. of methanol is refluxed for 2 hours.[1,2] The potassium chloride is filtered off, and the product is isolated by fractional vacuum distillation; yield 1.2 g. (80%), b.p. 108° (14 mm.).

(b) *3-Bromopropionic-1-C^{14} Acid.* A mixture of 2.5 g. (35.1 mmoles) of ethylene cyanohydrin-C^{14} and 25 ml. of 48% hydrobromic acid is refluxed for 2 hours.[1] The solution is cooled, diluted with 20 ml. of water and

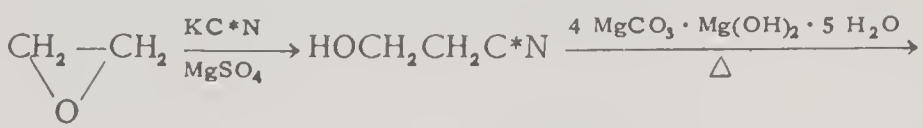
extracted with five 10-ml. portions of ether. The dried extract is concentrated and cooled, and the crude product is collected on a filter. Recrystallization from "isohexane" gives the purified product in 92% yield, m.p. 60–61°.

(c) *Ethyl 3-Bromopropionate-1-C^{14}.* A mixture of 5 g. (29.2 mmoles) of 3-bromopropionic-1-C^{14} acid, 25 ml. of absolute ethanol, 25 ml. of carbon tetrachloride and 0.2 g. of sulfosalicylic acid catalyst is subjected to slow distillation, such that the volume is reduced to one-half in 1.5 hours. Another 5 ml. of ethanol is added and distillation is continued. The product is finally isolated by vacuum distillation; yield 4.8 g. (88%), b.p. 88.5–90° (26 mm.).

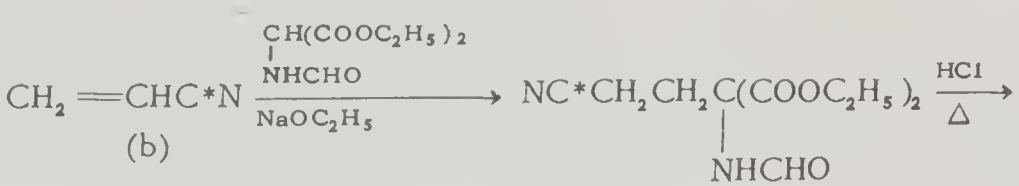
(d) *Glutamic-5-C^{14} Acid.* Ethyl phthalimidomalonate is condensed with ethyl 3-bromopropionate-1-C^{14}, and the condensation product is hydrolyzed according to the procedure of Marvel and Stoddard,[3] which follows. The yield is 47%, based on potassium cyanide-C^{14}.

To a solution of 2.7 g. (0.117 mole) of sodium in 53 ml. of absolute ethanol is added 30.5 g. (0.1 mole) of ethyl phthalimidomalonate and 14 g. (0.102 mole) of ethyl 3-chloropropionate. The mixture is refluxed for 9 hours, then concentrated under reduced pressure at 150°. The residue is extracted with two portions of hot water and then hydrolyzed by boiling for 12 hours with 75 ml. of concentrated hydrochloric acid and 25 ml. of water, 25 ml. of additional concentrated hydrochloric acid being added after 4 hours. The solution is cooled at 0° and filtered to remove phthalic acid, and the filtrate is evaporated to dryness under reduced pressure. The residue is dissolved in 40 ml. of water, and the solution is adjusted to pH 3 with concentrated ammonium hydroxide, seeded, cooled, and diluted with an equal volume of alcohol to crystallize the product. The yield of the monohydrate, after recrystallization from dilute alcohol, is 9.4 g. (57%).

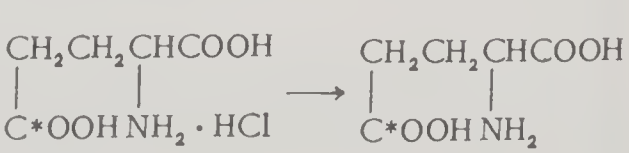
METHOD II



(a)



(b)



H. Tiedmann, Biochem. Z., *326*, 511 (1955).

A. Procedure

(a) *Hydracrylonitrile-1-C^{14}* (Note 1). To an ice-cooled solution of 0.618 g. of magnesium sulfate heptahydrate in 1 ml. of water is added 0.325 g. (5 mmoles) of potassium cyanide-C^{14} in 0.5 ml. of cold water, followed by 0.5 g. of cooled ethylene oxide. The mixture is shaken occasionally at 0° during 2 hours and stored at room temperature for 12 hours. Carbon dioxide is passed through the cooled suspension for 30 minutes; then the mixture is evaporated in a centrifuge cone at 30° under vacuum. The residue is extracted with three 4-ml. and eight 2-ml. portions of ethyl acetate. The combined extract is dried over calcium chloride and evaporated under vacuum.

(b) *Acrylonitrile-1-C^{14}*. To the crude hydracrylonitrile-1-C^{14} is added 0.030 g. of diethylene glycol and 0.030 g. of basic magnesium carbonate (Note 2), and the mixture is heated in a gentle stream of nitrogen with an air-bath preheated to 190°. The product passes through a jacketed-tube, maintained at the boiling point of isopropyl alcohol, and is condensed in a trap containing 0.08 g. of powdered anhydrous calcium chloride, and cooled by Dry Ice-chloroform; yield 0.160–0.185 g. (60–70%), b.p. 76–78°.

(c) *Glutamic-5-C^{14} Acid* (Note 3). To 1 g. of ethyl formamidomalonate (Note 4) is added 0.035 g. of sodium ethoxide and 2 ml. of absolute ether. The reaction flask is attached to a vacuum manifold, and 0.180 g. of acrylonitrile-1-C^{14} is added to the mixture by vacuum-transfer (liquid nitrogen) through a tube containing 2 cm. of finely granulated anhydrous calcium chloride. The mixture is shaken for 5 hours in a bath at 50°, stored for several hours at room temperature, acidified with dilute sulfuric acid and extracted with ether. The extract is dried and evaporated under vacuum, and the residue is heated for 6 hours at 115–120° with 10 ml. of concentrated hydrochloric acid. The mixture is evaporated under vacuum, and a concentrated aqueous solution of the residue is adjusted to pH 3 with 0.1 *N* sodium hydroxide and diluted with alcohol to precipitate the product. Purification is accomplished by shaking the crude product in 40 ml. of aqueous solution with 3 g. of Amberlite IR-45 (carbonate form) for 30 minutes (Note 5). The supernatant solution is treated with two 1-g. portions of the resin, and the resin is washed with two 10-ml. portions of water. The product is eluted by shaking the combined resin with 70 ml. of 0.2 *N* hydrochloric acid and with water. The combined eluate is evaporated under vacuum, the residue is dissolved in 50 ml. of water, and the solution is adjusted to pH 3.0 and concentrated to crystallize the product; yield 0.298 g. (60%).

B. Notes

1. The procedure is an adaptation of that of Terent'ev,[4] which gives hydracrylonitrile in yields of 85–90% at the 9-mole scale.

2. With larger amounts the cyanohydrin is added dropwise.

3. The method is that of Galat.[2] Directions are given[5] for preparing the starting ester.

4. Alternatively, 1.1 g. of ethyl acetamidomalonate may be used after the method of Lights.[6]

5. The procedure is designed to remove the glycine arising from unreacted ethyl formamidomalonate.

C. Other Preparations

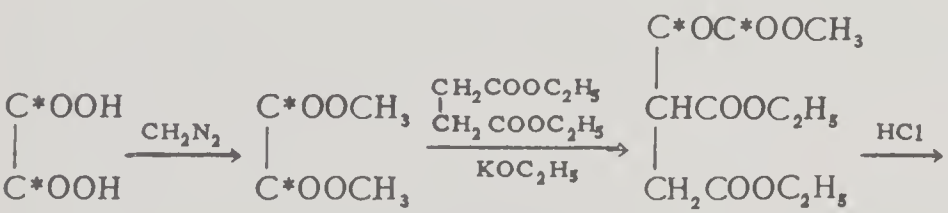
The preparation of hydracrylonitrile-1-C^{14} and 3-bromopropionic-1-C^{14} acid, by essentially Method I, has been reported by Martignoni.[7]

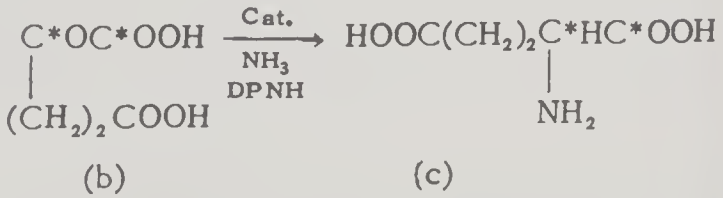
Depocas[8] has prepared glutamic-5-C^{14} acid by a modification of Method II (Note 4). Acrylonitrile-1-C^{14}, prepared from ethylene oxide, was converted to ethyl acetamido(2-cyano-C^{14}-ethyl)malonate which was hydrolyzed and decarboxylated in the described manner; also see 2-aminohexanedioic-6-C^{14} acid, Method II.

[1]W. A. Jacobs and M. Heidelberger, J. Am. Chem. Soc., *39*, 1465 (1917).
[2]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 256.
[3]C. S. Marvel and M. P. Stoddard, J. Org. Chem., *3*, 198 (1938).
[4]A. P. Terent'ev and E. V. Venogradova, J. Gen. Chem. (U.S.S.R.), *14*, 1044 (1944); Chem. Abstracts, *40*, 7157 (1946).
[5]A. Galat, J. Am. Chem. Soc., *69*, 965 (1947).
[6]L. Lights and Co. Ltd., and H. J. Weiss, Brit. 583,307; Chem. Abstracts, *41*, 2747 (1947).
[7]P. Martignoni and T. Winnick, J. Biol. Chem., *208*, 251 (1954).
[8]F. Depocas and L. P. Bouthillier, Rev. can. biol., *10*, 289 (1951); Chem. Abstracts, *45*, 9092 (1951).

L-GLUTAMIC-1,2-C_2^{14} ACID

METHOD I





R. W. Hendler, M. G. Horning and C. B. Anfinsen, Arch. Biochem. Biophys., *51*, 470 (1954).

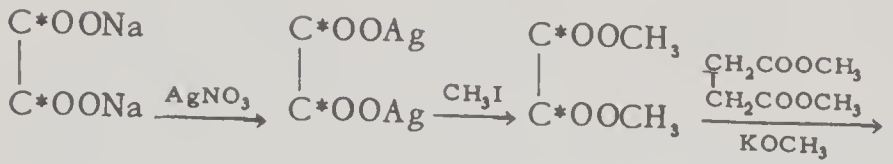
A. Procedure

(a) *Methyl Oxalate-C_2^{14}.* Oxalic-C_2^{14} acid (0.180 g., 2 mmoles) is esterified with diazomethane; yield 0.190 g. (100%), m.p. 53–53.5°.

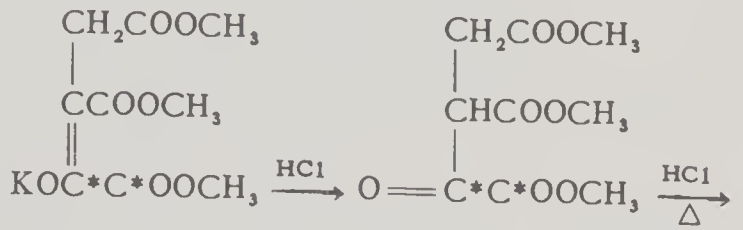
(b) *2-Oxoglutaric-1,2-C_2^{14} Acid* (Note 1). To a stirred solution of 0.075 g. (10% excess) of potassium in a solution of 0.28 ml. of absolute ethanol in 7 ml. of absolute ether, under nitrogen, is added 0.190 g. (1.6 mmoles) of methyl oxalate-C_2^{14} in a minimum of absolute ether. The mixture is stirred for 10 minutes, then is treated with 0.300 g. (10% excess) of ethyl succinate, and the stirring is continued for 25 minutes. The mixture is stored for 2.5 hours; then the potassium salt is collected and dissolved in 1.2 ml. of water and 0.2 ml. of concentrated hydrochloric acid. The ester is extracted into ether solution, the solvent is evaporated, and the residue is mixed with 1.0 ml. of concentrated hydrochloric acid and stored for 48 hours. The mineral acid is rapidly distilled off until the temperature reaches 140–150°; then the residue is cooled quickly and crystallized from benzene-ethyl acetate; yield 0.150 g., 64% based on oxalic acid.

(c) *L-Glutamic-1,2-C_2^{14} Acid.* To 6 ml. of a solution, 0.1 *M* in ammonium chloride and containing a sufficient amount of glutamic acid dehydrogenase (Note 2) and a slight excess of reduced diphosphopyridine nucleotide (Note 3), is added 0.005 g. (0.034 mmole) of 2-oxoglutaric-1,2-C_2^{14}. The reaction is stopped at about 45 minutes by diluting the mixture with 12 ml. of alcohol. The clear solution obtained by centrifuging is placed on a column of Dowex-50 (Note 4). The chromatogram is developed with 1.5 *N* hydrochloric acid, 3-ml. fractions being collected with a Technicon collector. Alternate tubes are assayed for activity (Note 5), and tubes 20–27 are pooled for the product cut (Note 6); radiochemical yield 79% (50.5% based on oxalic acid). The presence of only one isotopic compound is shown by paper chromatography and radioautography (Note 7). Degradation studies indicate that there is no isotopic rearrangement (Note 8), and the product is optically pure (Note 9).

METHOD II



(a)



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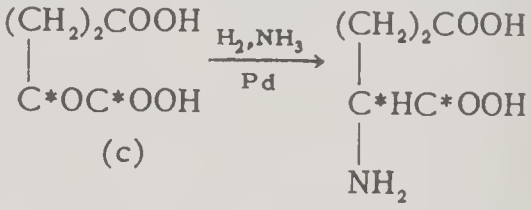


(c)

(d)

F. Kögl, J. Halberstadt and T. J. Barendregt, Rec. trav. chim., 68, 387 (1949).

A. Procedure

(a) *Methyl Oxalate-C_2^{14}.* A stirred mixture of 0.1144 g. of silver oxalate-C_2^{14} (Note 10) and 10 ml. of freshly distilled methyl iodide is refluxed for 8 hours in an apparatus protected from light with aluminum foil and fitted with a calcium chloride tube. The silver iodide is filtered off and washed with two 1-ml. portions of methyl iodide. The filtrate is concentrated at 0° in a stream of dry nitrogen; yield 0.0444 g., nearly quantitative (Note 11).

(b) *Methyl 3-Carbomethoxy-2-oxopotassiumglutarate-1,2-C_2^{14}, (Trimethyl Oxalyl-C_2^{14}-potassiumsuccinate).* Methyl succinate is condensed with 17.746 g. of methyl oxalate-C_2^{14} under nitrogen in the presence of potassium methoxide according to the procedure of Friedman and Kosower;[1] yield 30.2 g. (75%).

(c) *2-Oxoglutaric-1,2-C_2^{14} Acid* (Note 12). The potassium salt is dissolved in 41 ml. of water and acidified with 15 ml. of concentrated hydrochloric acid; then the free ester is extracted into ether. The ether is removed under vacuum, and the methyl 3-carbomethoxy-2-oxoglutarate-1,2-C_2^{14} (Note 13) is heated for 1 hour with 90 ml. of concentrated hydrochloric acid diluted with 200 ml. of water, then stored overnight. The turbid solution is filtered and evaporated at 50° under reduced pressure,

and the residual hydrochloric acid is removed by the evaporation of added water. The colorless crystalline product is dried under vacuum over potassium hydroxide; yield 14.21 g. (65%), m.p. 109° (Note 14).

(d) *Glutamic-1,2-C_2^{14} Acid.* The 2-oxoglutaric-1,2-C_2^{14} acid is hydrogenated, in three portions, over 1.25 g. of freshly prepared palladium oxide (Note 16) in 62 ml. of water and 17 ml. of 25% ammonium hydroxide. The hydrogenation is complete in about 8 hours of shaking; then the solution is filtered and evaporated to dryness under reduced pressure, and the residual ammonia is removed by the evaporation of additional water. The residue is dissolved in 12 ml. of water, concentrated hydrochloric acid is added to pH 3.3, and the mixture is stored in a refrigerator for 1 day. The product is collected, washed with a little ice water and dried under vacuum over potassium hydroxide; yield 9.863 g., 68.7% (Note 16).

(e) *Glutamic-1,2-C_2^{14} Acid Hydrochloride.* A solution of 3.23 g. of glutamic-1,2-C_2^{14} acid in 10 ml. of hot concentrated hydrochloric acid is filtered and stored in a refrigerator for 24 hours. The crystalline product is collected and washed with a few drops of hydrochloric acid (Note 17); yield 73.5%. The mother liquor yields further product after treatment with Norit. The product is recrystallized from 20% hydrochloric acid with 88.6% recovery (Note 18).

(f) *D-Glutamic-1,2-C_2^{14} Acid Hydrochloride.* A solution of 8.000 g. of DL-glutamic-1,2-C_2^{14} acid hydrochloride and 4.000 g. of D-glutamic acid hydrochloride in 200 ml. of hot 20% hydrochloric acid is cooled, seeded with the D-form and stored in a refrigerator for 24 hours. The collected product (4.75 g.) is recrystallized from 25 ml. of the hydrochloric acid; yield 4.10 g. (102.5%); $[\alpha]_D - 31.6°$.

(g) *L-Glutamic-1,2-C_2^{14} Acid Hydrochloride.* The combined mother liquors (f) are treated with 4.000 g. of L-glutamic acid hydrochloride; the mixture is adjusted to 200 ml. and worked up in the manner described above (Note 19); yield 4.10 g., $[\alpha]_D + 31.5°$.

B. Notes

1. The procedure is a modification of the method of Friedman and and Kosower.[1]

2. Directions are given by Olson[2] for preparing the enzyme solution from beef liver. The preparation (1/4 scale) is taken to the stage of dissolving the 28–40% sodium sulfate precipitate (fraction D_{28-40}) in phosphate buffer and bringing the sodium sulfate concentration back to 28%. This solution is dialized and used without further purification. Note: 0.05 *M* phosphate buffer is used in the initial step.

The enzyme activity is assayed[2] under the same reaction conditions in a nonisotopic experiment. The course of the reaction is followed by

withdrawing aliquots and determining the change in optical density at 340 mμ with a model DU Beckman spectrophotometer.

3. Directions are given by Olson[2,3] for preparing this DPNH from commercial preparations of 60% diphosphopyridine nucleotide. A solution is made up just before use containing 1 mg./ml. of 0.2 *M* potassium phosphate and adjusted to pH 7.6.

4. Dowex-50 (250–400 mesh, 12% divinyl benzene) is cycled through 4 *N* ammonium hydroxide and 6 *N* hydrochloric acid, then poured into a 1 × 46-cm. column and washed acid-free.

5. Aliquots (0.05 ml.) are dried on metal planchets and counted. A plot of activity vs. fraction number shows two distinct peaks.

6. The radiochemical recovery of unreacted 2-oxoglutaric-1,2-C_2^{14} acid (tubes 1–20) is 21% and in agreement with the equilibrium value.[3]

7. The crude product is contaminated with ammonia, which is removed by chromatography on Whatman No. 1 filter paper in 75% 1-propanol. The spot developed by spraying with ninhydrin agrees with the radioactive area and with the position for an authentic sample. The molar specific activity of the purified product is the same as that of the starting material.

8. Directions are given by Hendler[4] for decarboxylating with chloramine-T, forming succinaldehydic-4-C^{14} acid 2,4-dinitrophenylhydrazone, (C-2 to 5). Decarboxylation with *Clostridium welchii* cells forms 4-aminobutyric-4-C^{14} acid, (C-2 to 5), which is further decarboxylated by the Schmidt reaction, forming propanediamine-C^{14} dipicrate (C-2 to 4).

9. The specific activity of the 4-aminobutyric-4-C^{14} acid formed by decarboxylation with *Clostridium welchii* (specific for the L-form of glutamic acid) is 51% of that of the product glutamic acid.

10. The silver salt is precipitated by treating a warm solution of sodium oxalate-C_2^{14}, weakly acidified with acetic acid, with 1.5% silver nitrate solution. Silver oxalate-C_2^{14} is prepared[5] from the insoluble calcium salt by heating an aqueous suspension for 30 minutes just below a boil with ion exchange resin IR-100H, which has been freshly treated with dilute sulfuric acid. The hot solution is filtered, with thorough washing of the residue with hot water; then the product is precipitated by the addition of silver nitrate to the filtrate.

11. Speer[5] has prepared the product in 70% yield by this procedure; the purity was 95% on the basis of melting point and saponification equivalent.

12. The procedure is that of Friedman and Kosower.[1]

13. The ester may be purified by distillation at about 115° (1 mm.).

14. Speer[5] has prepared the product in 60–70% yield based on methyl oxalate, by employing ethyl succinate. The compound may be recrystallized from acetone-benzene. A degradation is described by Mosbach,[6] which avoids the formation of symmetrical products. The isotopic prepa-

ration by the described method from sodium formate-C^{14} has been reported by Wilson.[7]

15. A small amount of freshly reduced palladium-black is added to facilitate the start of the reduction. The preparation of palladium catalysts is described by Starr and Hixon[8] and by Shriner.[9]

16. The yield of individual runs was 64, 76 and 66%. Speer[5] has prepared the product (64% yield) by this method, but with a modified isolation procedure. The filtered hydrogenation solution was concentrated to 5 ml., made up to 25 ml. of 6 *N* hydrochloric acid solution, and refluxed for 8 hours at 100–105°. The solution was concentrated under vacuum to 5 ml., adjusted to pH 3.2 with aniline, then diluted with ethanol until the mixture was 90% alcohol and stored in a refrigerator to crystallize the product. The preparation has been described by Mosbach;[6] yield 60–80%. Degradations are described by Mosbach,[6] Wang,[10] Miller,[11] Kögl[12] and Ehrensvärd;[13] also see Note 8.

17. The acid is saturated with hydrogen chloride at 0°.

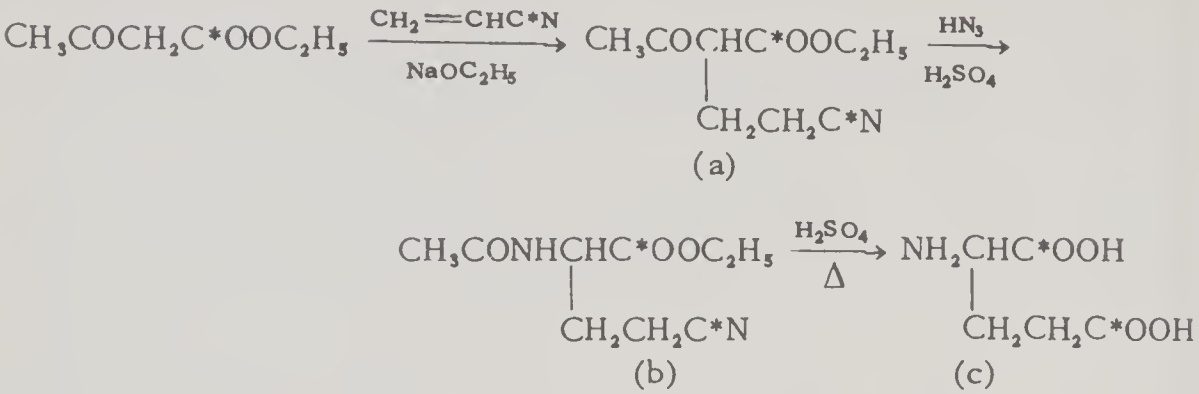
18. The activity is divided equally between the D- and L-forms, as determined by assay of the resolved products. The problem of analysis by isotope dilution is discussed by Rittenberg.[14,15]

A degradation of C^{14}-glutamic acid is described by Miller;[11] ninhydrin liberates the 1-carboxyl as carbon dioxide, while the Schmidt reaction liberates the 5-carboxyl as carbon dioxide, forming 2,4-diaminobutyric acid (65–70%).

19. The solubility of the DL-component in 5.4 *N* hydrochloric acid at 0° is 2.48 g./100 g. of solution, twice that of the L-component.[14]

[1]*Organic Syntheses*, Vol. 26, Wiley, New York, 1946, p. 42.
[2]J. A. Olson and C. B. Anfinsen, J. Biol. Chem., *197*, 67 (1952).
[3]J. A. Olson and C. B. Anfinsen, J. Biol. Chem., *202*, 841 (1953).
[4]R. W. Hendler and C. B. Anfinsen, J. Biol. Chem., *209*, 55 (1954); H. D. Dakin, Biochem. J., *11*, 79 (1917).
[5]R. J. Speer, A. Roberts, M. Maloney and H. R. Mahler, J. Am. Chem. Soc., *74*, 2444 (1952).
[6]E. H. Mosbach, E. F. Phares and S. F. Carson, Arch. Biochem. Biophys., *33*, 179 (1951).
[7]D. G. Wilson, K. W. King and R. H. Burris, J. Biol. Chem., *208*, 863 (1954).
[8]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 566.
[9]R. L. Shriner and R. Adams, J. Am. Chem. Soc., *46*, 1683 (1924).
[10]C. H. Wang, B. E. Christensen and V. H. Cheldelin, J. Biol. Chem., *201*, 683 (1953).
[11]L. L. Miller and W. F. Bale, Arch. Biochem., *48*, 361 (1954).
[12]F. Kögl, P. Emmelot and D. H. W. den Boer, Ann., *589*, 1 (1954).
[13]G. Ehrensvärd, L. Reio, E. Saluste and R. Stjernholm, J. Biol. Chem., *189*, 93 (1951).
[14]S. Graff, D. Rittenberg and G. L. Foster, J. Biol. Chem., *133*, 745 (1940).
[15]D. Rittenberg and G. L. Foster, J. Biol. Chem., *133*, 737 (1940).

GLUTAMIC-1,5-C_2^{14} ACID



H. Tiedmann, Biochem. Z., *326*, 511 (1955).

A. Procedure

(a) *Ethyl 2-(2-Cyano-C^{14}-ethyl)acetoacetate-1-C^{14}.* To a stirred mixture of 0.050 g. of sodium in 0.5 ml. of absolute alcohol, 1.5 ml. of ether and 2.17 g. (16.7 mmoles) of ethyl acetoacetate-1-C^{14} is added dropwise 0.89 g. (1.1 ml.) of acrylonitrile-1-C^{14}. The mixture is heated for 3 hours at 70–80°, cooled, acidified with 2 *N* sulfuric acid and extracted with ether. The extract is dried over calcium chloride and evaporated under vacuum. The residue is fractionally distilled; yield 1.66 g. (54%), b.p. 157–158° (17 mm.) (Note 1).

(b) *Ethyl 2-Acetamido-4-cyano-C^{14}-butyrate-1-C^{14}* (Note 2). To a stirred ice-cooled mixture of 3.2 ml. of concentrated sulfuric acid and 2 ml. of chloroform is added, dropwise during 1 hour, a solution containing the ester, 1.5 ml. of chloroform and 5.9 ml. of 1.7 *N* hydrazoic acid in chloroform (Note 3). After 7 hours the chloroform layer is separated and extracted with two 1-ml. portions of sulfuric acid. The combined acid is poured onto 15 g. of ice (Note 4).

(c) *Glutamic-1,5-C_2^{14} Acid.* The mixture of the amide-ester and dilute sulfuric acid is heated at 110–120° for 5 hours, cooled to 50° and treated with a solution of about 30 g. of barium hydroxide octahydrate in 100 ml. of water to remove all sulfate ions (Note 5). The precipitate is separated by centrifuging and washed with four 100-ml. portions of warm water. The combined aqueous solution is concentrated under reduced pressure and diluted with alcohol to crystallize the product; yield 1.015 g. (76%). Purification is accomplished by recrystallization from dilute alcohol, m.p. 197°. Paper chromatography (80% phenol) demonstrates the presence of trace amounts of glycine (Note 6).

B. Notes

1. Unreacted ethyl acetoacetate-1-C^{14} is recovered, 0.7 g.
2. The Schmidt reaction is surveyed by Wolff.[1]

3. Directions are given by Wolff[1] for preparing and standardizing the reagent.

4. The precipitated oil may be crystallized by trituration with water.

5. The final pH is about 3.

6. The contaminant may be removed by the procedure described for the purification of glutamic-5-C^{14} acid, Method II.

[1]*Organic Reactions*, Vol. III, Wiley, New York, 1946, p. 307.

GLUTAMIC-3,4-C_2^{14} ACID

$$HC^*\equiv C^*H \xrightarrow[Ni(CO)_4,\ HCl]{CH_3OH} C^*H_2{=}C^*HCOOCH_3 \xrightarrow[NaOC_2H_5]{CH(COOC_2H_5)_2,\ NHCOCH_3} \quad \text{(a)}$$

$$C^*H_2C^*H_2CH(COOC_2H_5)_2,\ COOCH_3,\ NHCOCH_3 \xrightarrow[\Delta]{HCl} C^*H_2C^*H_2CHCOOH,\ COOH,\ NH_2 \quad \text{(b)}$$

L. Pichat, C. Baret, M. Audinot, M. Herbert and J. Lambin, *Radioisotope Conference. 1954*, Vol. I, Academic Press, New York, 1954, p. 245.

A. Procedure

(a) *Methyl Acrylate-2,3-C_2^{14}* (Note 1). A mixture of 0.265 ml. of nickel carbonyl, 0.85 ml. of methanol and 0.41 ml. of concentrated hydrochloric acid is frozen in a 500-ml. round-bottomed flask, and 9.15 mmoles of acetylene-C_2^{14} is added by vacuum-transfer. The flask is closed, detached from the manifold and shaken at 60° for 3 hours. The crude product, after removal of condensable gases (Note 2), is vacuum-distilled (Note 3) onto powdered calcium oxide and stored for 3 hours to dry (Note 4). The purified product is collected by vacuum distillation (Note 5); yield 50% based on acetylene.

(b) *Glutamic-3,4-C_2^{14} Acid* (Note 6). To a frozen mixture of 1.0 g. of ethyl acetamidomalonate and 0.0075 g. of sodium in 3 ml. of absolute alcohol is added the methyl acrylate-2,3-C_2^{14} by vacuum distillation at room temperature. The flask is sealed and shaken at 90° for 9 hours. The solvent is removed under vacuum at room temperature (Note 7), and the residue is refluxed with 4 ml. of 6 *N* hydrochloric acid, while 4 ml. of concentrated hydrochloric acid is added during 1 hour. The heating is continued for 6 hours, and the mixture is evaporated under vacuum. The residue is dissolved in 1 ml. of 8 *N* ammonium hydroxide, and the solution

is diluted with 4 ml. of water and 10 ml. of absolute alcohol, then adjusted to pH 3.3 with concentrated ammonium hydroxide and stored at 0° for 2 days. The product monohydrate) is collected; yield 0.463 g. (34.5% based on acetylene). Paper chromatography indicates that the product contains 5% inactive glycine and no other detectable impurities.

B. Notes

1. The procedure is an adaptation of the "stoichiometric method" of Reppe.[1] Hanford[2] has reported a yield of methyl acrylate of 78.7% based on nickel carbonyl and 36% based on acetylene; Reppe, on the other hand, reported a yield of 80% on either basis. The distribution of radioactivity in most of the residues attests to the complex nature of Reppe's reaction.

2. Condensable gases at the end of the reaction account for 5% of the initial activity.

3. The residue contains 20% of the initial activity.

4. Methyl acrylate, b.p. 30° (150 mm.), forms an azeotrope with water.

5. The residue contains 8% of the initial activity. The yield may be determined in trial preparations, by the hydrogenation method[3] over Raney nickel.

6. The procedure is an adaptation of that of Snyder.[4]

7. The alcohol contains 3.5% of the initial activity.

C. Other Preparations

The preparation of methyl acrylate-2,3-C_2^{14} by a two-step process, involving critical steps, has been studied in detail by Pichat. Hydracrylonitrile-2,3-C_2^{14} was formed (yield 83–89%, n_D^{20} 1.427–1.422) by storing a mixture of 5 mmoles of ethylene-C_2^{14} oxide, 5 mmoles of hydrogen cyanide, 0.003 g. of potassium cyanide catalyst[5] and 100 ml. of water for 5 days at 24° in a sealed tube under vacuum. A mixture of 0.500 g. of hydracrylonitrile-2,3-C_2^{14}, 0.400 g. of methanol and 1 ml. of concentrated sulfuric acid is shaken for 2.5 hours at 140° in a sealed tube under vacuum.[6] The mixture is vacuum-distilled, giving a 70% methanolic solution of the ester; yield 90–96% (based on refractive index of known mixtures), up to 54% based on acetylene.

Acrylonitrile-2,3-C_2^{14} has been prepared by the liquid phase catalytic dehydration, of hydracrylonitrile-2,3-C_2^{14} at 220° over 10%, by weight, of sodium formate. The product was dried over calcium chloride and distilled; yield 87%, n_D^{22} 1.389.

[1]W. Reppe, A. Magin, C. Schuster, R. Keller, H. Kröper, T. Klein, F. W. Kerckow, G. V. Blank, K. Merkl, H. Scheller, L. Weschky, K. Wolff, W. Schwecken-

diek, O. Hecht, E. Gassenmeier and A. Simon, Ann., *582*, 1 (1953); E. R. H. Jones, T. Y. Shen and H. C. Whiting, J. Chem. Soc., *1951*, 769.

[2]W. E. Hanford and D. L. Fuller, Chem. Eng. News, *40*, 1171 (1948).

[3]S. Siggia, *Quantitative Analysis via Functional Groups*, Chapman and Hall, London, *1949*, p. 37.

[4]H. R. Snyder, J. F Shekleton and C. D. Lewis, J. Am. Chem. Soc., *67*, 310 (1945).

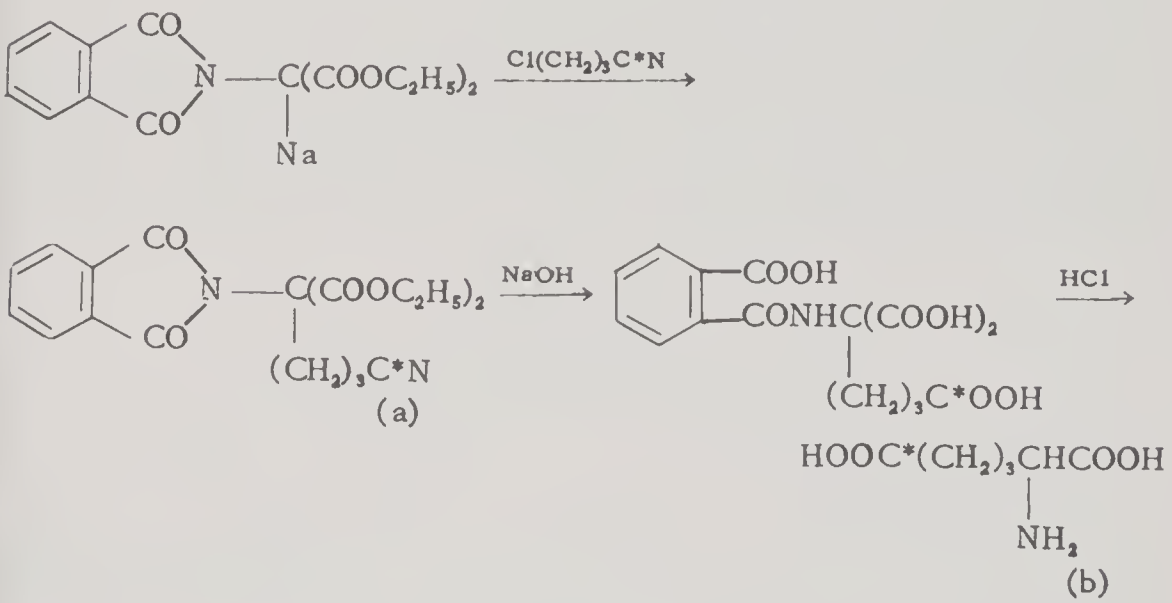
[5]E. Rambaud, Bull. soc. chim. France, (5) *3*, 138 (1936).

[6]W. Bauer, U. S. 1,829, 208; Chem. Abstracts, *26*, 735 (1932).

[7]E. L. Carpenter, U. S. 2,494,116; Chem. Abstracts, *44*, 3006 (1950).

2-AMINOHEXANEDIOIC-6-C^{14} ACID
(2-Aminoadipic-6-C^{14} Acid)

METHOD I



H. Borsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighley and P. H. Lowy, J. Biol. Chem., *176*, 1395, 1383 (1948).

A. Procedure

(a) *Ethyl* α-*(3-Cyano-*C^{14}*-propyl)-1,3-dioxo-2-isoindolinemalonate*, [*Ethyl (3-Cyano-*C^{14}*-propyl)phthalimidomalonate*]. The isotopic preparation is made from 4-chlorobutyronitrile-1-C^{14} by suitable modification of the non-isotopic synthesis, which follows.

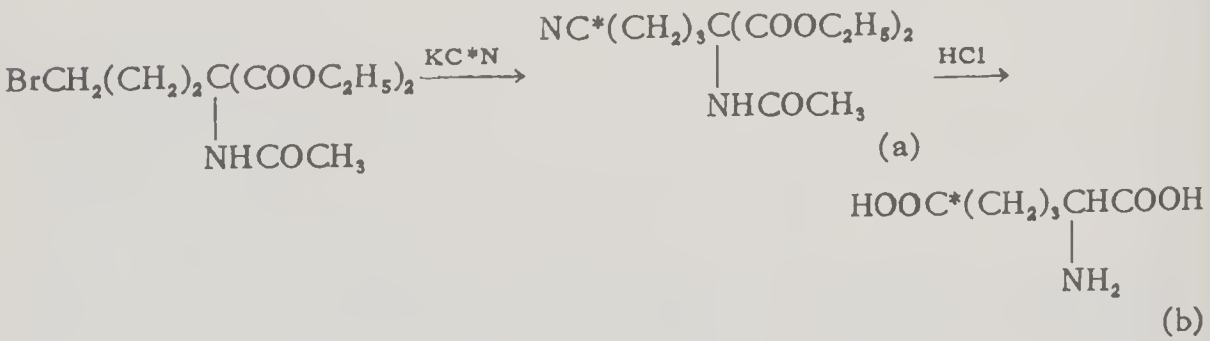
A mixture of 1.228 g. of 4-chlorobutyronitrile and 2.060 g. of ethyl sodiumphthalimidomalonate[1] is heated under reflux at 160–165° for 4 hours, at which time the alkaline reaction to litmus has disappeared. The ex-

cess nitrile is removed by steam distillation, the residue is cooled in ice, and the product is collected, washed with water by trituration and dried; yield 2.050 g.

(b) *2-Aminohexanedioic-6-C^{14} Acid.* A mixture of the nitrile, 12.5 ml. of absolute ethanol and 11 ml. of 4 *N* sodium hydroxide is refluxed for 3 hours. Concentrated hydrochloric acid (16 ml.) is added, and heating is continued for 3 hours. The solution is evaporated on a steam-bath, and the residue is cooled in ice and extracted by repeated washing with ice-cold 33% hydrochloric acid. The filtered solution is evaporated and dried over sulfuric acid and sodium hydroxide; the yield of crude 2-aminohexanedioic-6-C^{14} acid hydrochloride is 1.206 g. The salt is dissolved in 2.5 ml. of water and filtered into a 10-ml. beaker by washing with 1.3 ml. of water. The filtrate is adjusted to pH 3.1, with 5 *N* ammonium hydroxide, then set aside to crystallize the product, which is collected and washed with a little water, alcohol and ether. The yield is 0.629 g., 62% based on ethyl sodiumphthalimidomalonate.

The product is resolved by the carbobenzyloxy-anilide-papain method of Bergmann (see L-lysine-6-C^{14}). *N*-Carbobenzyloxy-DL-2-aminohexanedioic-6-C^{14} acid melts at 124°, and *N*-carbobenzyloxy-L-2-aminohexanedioic-6-C^{14} acid anilide melts at 170–171°. L-2-Aminohexanedioic-6-C^{14} acid melts at 205° (dec.), $[\alpha]_D^{25}$ + 33.9°, (*c* 5.49, 6 *N* HCl) (Note 1).

METHOD II



M. Rothstein and C. J. Claus, J. Am. Chem. Soc., 75, 2981 (1953).

A. Procedure

(a) *Ethyl Acetamido(3-cyano-C^{14}-propyl)malonate.* A solution of 0.400 g. of potassium cyanide-C^{14} in 7.4 ml. of water (including 2 ml. of wash water) is added to a solution of 2 g. of ethyl acetamido(3-bromopropyl)malonate (Note 2) in 20 ml. of ethanol. The mixture is refluxed for 2.5 hours (Note 2), then taken to dryness by distillation under reduced pressure (Note 3). The residue is extracted with several portions of

ether (Note 4), and the extracts are dried and evaporated to yield the product as a dark oil (Note 5).

(b) *2-Aminohexanedioic-6-C^{14} Acid.* The crude nitrile is hydrolyzed by refluxing for 5 hours with 30 ml. of concentrated hydrochloric acid. The solution is distilled to dryness under reduced pressure, water is added, and the distillation is repeated until there is left a crystalline residue, which is then dissolved in a small volume of water. The volume of filtered solution is made up to 12 ml., and 15 ml. of alcohol is added; the solution is mixed with a slight excess of pyridine and left overnight in a refrigerator. The colorless crystalline product is collected, washed halogen-free with alcohol and dried; yield 0.389 g. (41%). Filter paper chromatography shows the presence of only one compound (Note 6).

B. Notes

1. A resolution by the asymmetric enzymatic hydrolytic method of Greenstein[2] for DL-α-amino acids (see D-alanine-1-C^{14}) has been reported by Rothstein;[3] L-2-aminohexanedioic acid, $[\alpha]_D^{25}$ + 25.0° (2% in 5 *N* HCl).

2. The starting material may be prepared[4] in 68% yield by brominating the corresponding carbinol with phosphorus tribromide. The carbinol is prepared[5] by the 1,4-addition of ethyl acetamidomalonate and acrolein (87% yield), followed by reduction of the ethyl acetamido(2-formylethyl)malonate over Raney nickel catalyst (82% yield).

3. The evolved hydrogen cyanide-C^{14} (45%) is absorbed in a trap containing 35 ml. of carbonate-free 1.5 *N* sodium hydroxide.

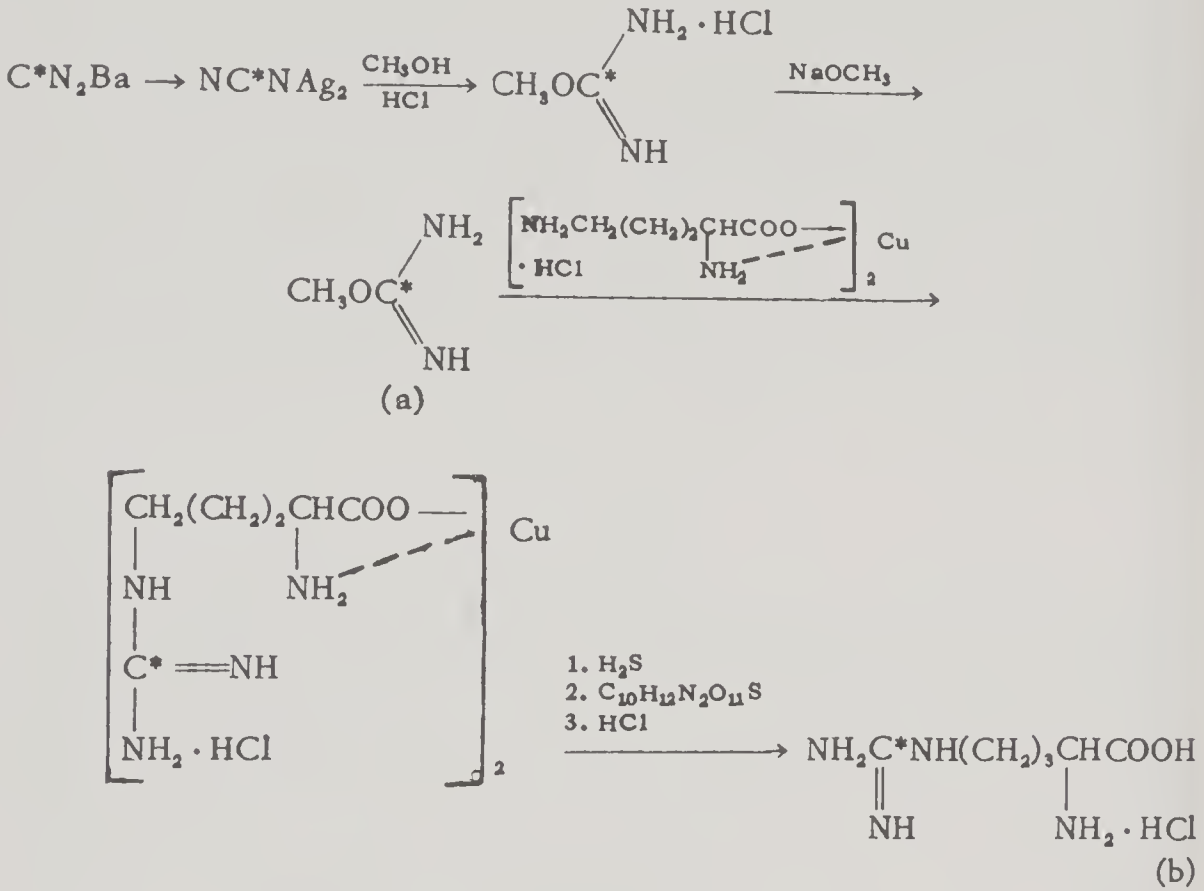
4. The residue of potassium bromide (0.73 g.) corresponds to a quantitative conversion.

5. As the product could not be crystallized, optimum conditions for cyanation were determined by hydrolysis to the crystalline acid (b). Shorter or longer periods of heating decrease the yield of 44% (Note 6). The yield is not improved by employing a 25% excess of the bromoester. The yield is lowered by using methanol or ethylene glycol as solvent, or by the dropwise addition of either reactant.

6. The yield is about 75%, if correction is introduced for the recovered cyanide-C^{14}. The 2-benzamidohexanedioic acid melts at 183.5–184.5°.

[1] *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 384.
[2] J. P. Greenstein, S. M. Birnbaum and M. C. Otey, J. Am. Chem. Soc., 75, 1994 (1953).
[3] M. Rothstein and L. L. Miller, J. Biol. Chem., *211*, 851 (1954).
[4] M. Rothstein and C. J. Claus, J. Am. Chem. Soc., 75, 2981 (1953).
[5] O. A. Moe and D. T. Warner, J. Am. Chem. Soc., 70, 2763 (1948).

2-AMINO-5-GUANIDINO-C^{14}-VALERIC ACID HYDROCHLORIDE (C_1^{14}-L-Arginine Hydrochloride)



F. Turba and A. Leismann, Angew. Chem., 65, 535 (1953).

A. Procedure

(a) *2-Methylpseudourea-C^{14}*, (*O-Methylisourea-C^{14}*) (Note 1). Barium carbonate-C^{14} (0.160 g.) is heated at 800° for 6 hours in a stream of dry ammonia. The residue is dissolved in dilute acetic acid, and the solution is treated with an excess of silver nitrate and ammonia, precipitating the silver cyanamide-C^{14} which is collected in quantitative yield. A mixture of the dry silver compound and 0.65 ml. of methanolic hydrogen chloride (Note 2) is stored in the dark for 14 days. The precipitate is separated by centrifuging and washed with absolute methanol under anhydrous conditions. From the combined solutions is crystallized 2-methylpseudourea-C^{14} hydrochloride as thick crystals; yield 0.0745 g. (Note 3). The free base is liberated by adding a solution of 0.0173 g. of sodium in absolute methanol and separating the sodium chloride by centrifuging.

(b) *2-Amino-5-guanidino-C^{14}-valeric Acid Hydrochloride*, (*C_1^{14}-L-Arginine Hydrochloride*) (Note 4). To the methanol solution of 2-methylpseudo-

urea-C^{14} is added a solution of 0.161 g. of L-argininecopper chloride (Note 5) in a little water, followed by the free base from 0.021 g. of 2-methylpseudourea-C^{14} hydrochloride. At 24 hours the free base from 0.050 g. of the hydrochloride is added. At 48 hours the mixture is acidified with *N* sulfuric acid (Congo red) and saturated with hydrogen sulfide to remove copper. To the hot filtered solution is added 0.500 g. of flavianic acid, and the C_1^{14}-L-arginine flavianate is crystallized by cooling. The glittering yellow flakes are separated by centrifuging, then washed with a little water and recrystallized from methanol at 0°; yield 0.230 g. The flavianate is converted to the product by dissolution in dilute hydrochloric acid, extraction with amyl alcohol-benzene (1: 1), and evaporation of the colorless solution. The over-all yield is 80% based on barium carbonate. The product is shown to be pure by paper electrophoresis and radioautography (Note 6).

B. Notes

1. The procedure is adapted from that of Rodionov;[1] also see barium and silver cyanamide-C^{14}.

2. Dry hydrogen chloride is passed through 4.57 g. of methanol until the weight increases to 6.02 g.

3. 2-Methylpseudourea-C^{14} hydrochloride[2] may be recrystallized from methanol, m.p. 122–124° (dec.), and stored safely in small filled vials in a desiccator. The salt is decomposed in warm aqueous solution, forming chloromethane and urea.

4. The procedure is adapted from that described by Turba.[3]

5. L-Ornithine is prepared from L-arginine by refluxing for 1 hour with saturated barium hydroxide solution, and then is precipitated as the benzylideneornithine-copper complex by treatment with copper chloride and benzaldehyde as described by Turba[4] for lysine; also see Brand and and Sandberg.[5]

6. Pyridine-acetate buffer, pH 6.5; 40 volts/cm.; 10°; 2 hours; 1% ninhydrin in butanol; Sakaguchi reagent. Pure arginine hydrochloride sinters at 218°, solidifies again at 225° and decomposes at 235°.

[1]V. M. Rodionov and O. S. Urbanskaya, J. Gen. Chem. U.S.S.R., *18*, 2023 (1948); Chem. Abstracts, *43*, 3793 (1949).

[2]*Organic Syntheses*, Vol. 34, Wiley, New York, 1954, p. 67.

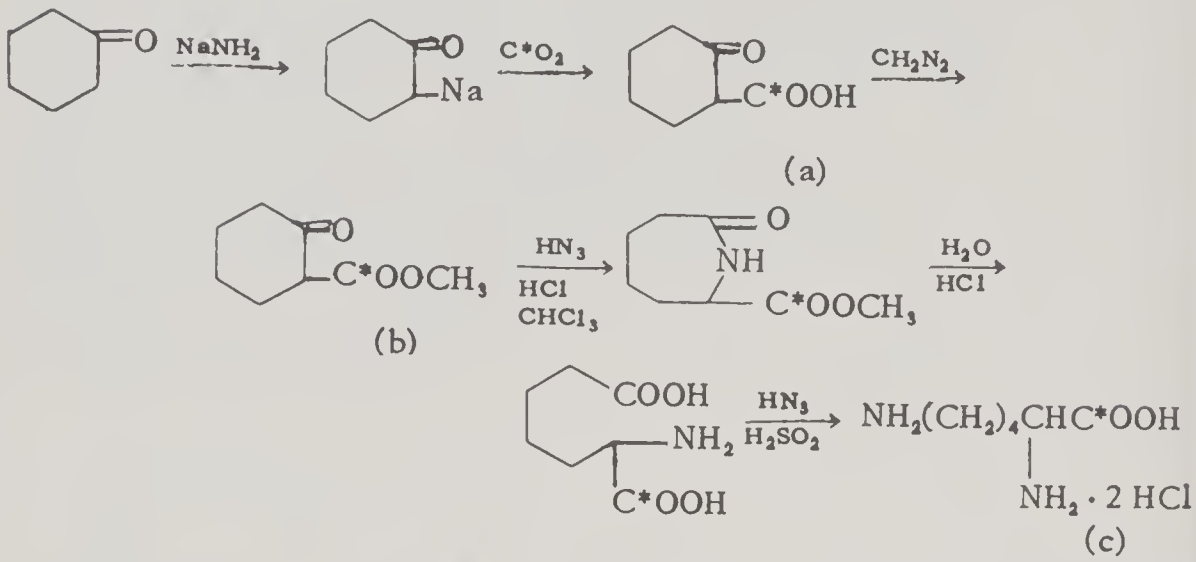
[3]F. Turba and K. Schuster, Hoppe-Seyler's Z. physiol. Chem., *283*, 27 (1948).

[4]F. Turba, *ibid.*, *283*, 19 (1948).

[5]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 49.

LYSINE-1-C^{14} DIHYDROCHLORIDE
(2,6-Diaminohexanoic-1-C^{14} Acid Dihydrochloride)

METHOD I



H. R. V. Arnstein, G. D. Hunter, H. M. Muir and A. Neuberger, J. Chem. Soc., *1952*, 1329.

A. Procedure (Note 1)

(a) *2-Oxocyclohexanecarboxylic-C^{14} Acid* (Note 2). A solution of 5.0 g. of cyclohexanone and 30 ml. of absolute ether is added during 5 minutes to a stirred solution of sodamide, prepared from 1.4 g. of sodium in 50 ml. of anhydrous liquid ammonia (Note 3). Ammonia is distilled off as quickly as possible and ether is added to maintain a volume of about 60 ml. The mercury-sealed stirrer is replaced by an induction stirrer, the condenser and dropping funnel are removed, and the flask is attached to the manifold of a high-vacuum system. The mixture is frozen in liquid nitrogen, and the system is evacuated to 1 μ, then isolated from the pump. The stirred mixture is carbonated at -15° with the carbon-C^{14} dioxide from 11.0 g. of barium carbonate-C^{14}. After one hour, the excess carbon-C^{14} dioxide is removed (Note 4) and the minimum amount of water is added to effect a separation of the ether phase. The aqueous layer is transferred to a three-necked flask equipped with a dropping funnel, a gas inlet tube and an outlet connected to the alkali traps. Ice-cold 20% sulfuric acid (50% excess) is added dropwise to the cooled solution while a slow stream of nitrogen carries the evolved carbon-C^{14} dioxide into the absorbers (Note 5). The product is extracted into ether.

(b) *Methyl 2-Oxocyclohexanecarboxylate-C^{14}*. 2-Oxocyclohexanecarboxylic-C^{14} acid is esterified by the procedure of Levine and Hauser,[1] which follows. The yield of distilled product is 3.0 g.

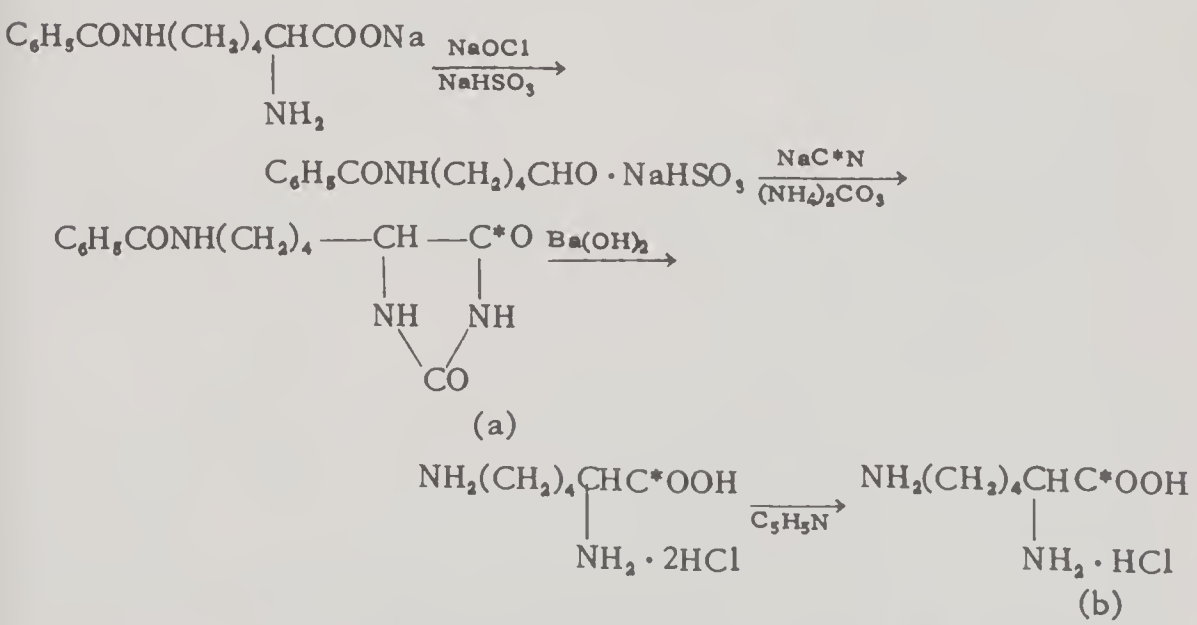
An ether extract of the β-keto acid is dried with sodium sulfate at 0° for 30 minutes, then treated with a 10–20% excess of diazomethane in

ether. The excess reagent is destroyed by addition of a slight excess of glacial acetic acid (Note 6). The ether solution is washed with saturated sodium bicarbonate, then dried over Drierite, concentrated and fractionally vacuum-distilled (Note 7).

(c) *Lysine-1-C^{14} Dihydrochloride.* The methyl 2-oxocyclohexanecarboxylate-C^{14} is converted to the product by the method of Adamson,[2] which follows. The over-all isotopic yield is 12.2% based on barium carbonate-C^{14}.

A mixture of 28.4 g. of ethyl 2-oxocyclohexanecarboxylate, 125 ml. of chloroform and 8 g. (15% excess) of hydrazoic acid (Note 8) is stirred, below 5°, in a stream of hydrogen chloride for 3 houra. Concentrated hydrochloric acid (150 ml.) is added, and the aqueous layer is separated, diluted with an equal volume of water and refluxed for 2 hours. The mixture is evaporated under reduced pressure, and the residue (Note 9) is taken up in 60 ml. of concentrated sulfuric acid and 60 ml. of chloroform at 45°. A solution of 8 g. of hydrazoic acid in 75 ml. of chloroform is added in 5-ml. portions during 6 hours. After the evolution of nitrogen and carbon dioxide (8 hours), the mixture is stirred overnight at room temperature, then poured over ice. The aqueous layer is separated and hot barium hydroxide solution is added until the mixture is only weakly acidic. Barium sulfate is removed by centrifugation, a hot solution of 53 g. of picric acid in 1 l. of water is added to the supernatant liquid (1.2 l.) and the mixture is stored in a refrigerator overnight. The yield of lysine dipicrate, dried in a vacuum over sulfuric acid, is 60.5 g. (60%), m.p. 184–187°. Decomposition by hydrochloric acid, and ether extraction gives the pure dihydrochloride in 19.6 g. yield, 54% (Note 10).

METHOD II



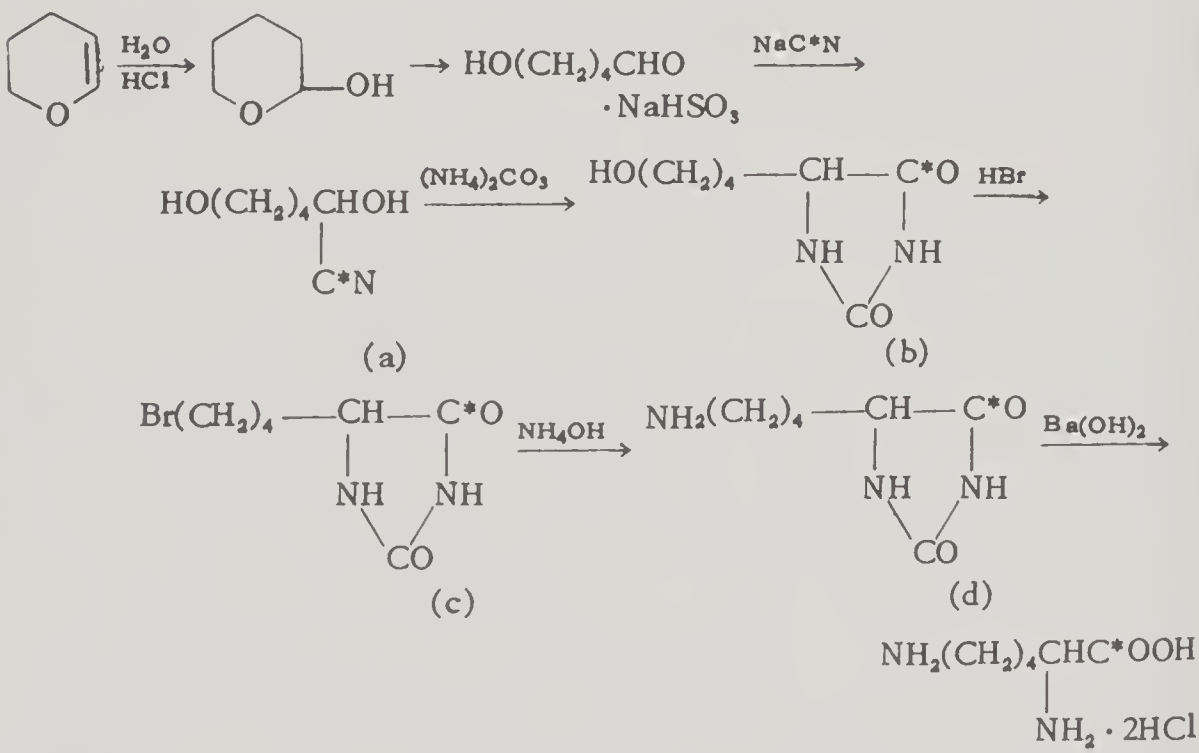
V. I. Maimind, B. V. Tokarev and M. M. Shemyakin, Doklady Akad. Nauk S.S.S.R., 92, 81 (1953); through Chem. Abstracts, 48, 10588 (1954).

A. Procedure

(a) *5-(4-Benzamidobutyl)hydantoin-4-C*^{14}. A mixture of 1.8 g. of crude 5-benzamidovaleraldehyde sodium bisulfite adduct (Note 11), 0.9 g. of ammonium carbonate, 2 ml. of aqueous sodium cyanide-C^{14} (2.7 mmoles) and 1.5 ml. of alcohol is heated at 100° for 1 hour in a sealed tube (shaking). The mixture is evaporated on a steam-bath (20–30 minutes), and the residue is acidified with concentrated hydrochloric acid (Congo red), heated on a steam-bath for 5 minutes and stored for several hours at 0°. The product is collected on a filter, washed with 8–10 ml. of water, triturated with ethyl acetate, washed with ether and dried; yield 70%, m.p. 186–186.5° from alcohol.

(b) *Lysine-1-C*^{14} *Monohydrochloride*. A mixture of 0.520 g. of the hydantoin, 2 g. of crystalline barium hydroxide and 10 ml. of water is heated for 1 hour in a sealed tube at 160–165°, then saturated with carbon dioxide and heated for 3–5 minutes on a steam-bath. The filtered solution is concentrated under vacuum at 40–45°, diluted with 15 ml. of water, acidified with *N*-hydrochloric acid, filtered to remove benzoic acid and evaporated under vacuum. The syrup is diluted with 6 ml. of hot alcohol, followed by 60 ml. of acetone, and stored at 0° for 2 days. The dihydrochloride is collected and converted to the monohydrochloride (Note 12); yield 81%, m.p. 255–256° from dilute alcohol.

METHOD III



H. Borsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighley and P. H. Lowy, J. Biol. Chem., *184*, 529 (1950).

A. Procedure

The preparation is by the method of Gaudry,[3] which follows, suitably modified to adapt it to a small scale (Note 13). The yield of lysine-1-C^{14} monopicrate, from 10 mmoles of sodium cyanide-C^{14}, is 10–15%.

(a) *2,6-Dihydroxyhexanenitrile-1-C^{14}.* The aqueous solution of 5-hydroxyvaleraldehyde bisulfite addition compound prepared from 15 mmoles of dihydropyran (Note 14) is added to a flask containing 10 mmoles of sodium cyanide-C^{14} and stirred for 2 hours at room temperature. The mixture is extracted with liberal amounts of ether, and the ether is evaporated, leaving the product as a colorless liquid.

(b) *5-(4-Hydroxybutyl)hydantoin-4-C^{14}.* A mixture of the crude nitrile and an excess of aqueous ammonium carbonate[4] is stirred for 1 hour at 50–55°. The bath temperature is raised slowly to 100°; then the solution is decolorized with charcoal and evaporated to dryness. The residue is crystallized from water, m.p. 152–155° (Note 15).

(c) *5-(4-Bromobutyl)hydantoin-4-C^{14}.* 5-(4-Hydroxybutyl)hydantoin (34.4 g., 0.2 mole) is treated with 400 ml. of 48% hydrobromic acid. The solution is heated at 90° for 2 hours, then evaporated to dryness under reduced pressure. The residue is decolorized in aqueous solution with charcoal, and the filtrate is cooled, refiltered and evaporated to dryness. The residue is treated again with 100 ml. of hydrobromic acid as before. The product is crystallized from 30 ml. of hot water; yield 38.8 g. (80%), m.p. 129–131° after two crystallizations.

(d) *5-(4-Aminobutyl)hydantoin-4-C^{14}.* A mixture of 7.83 g. (33 mmoles) of 5-(4-bromobutyl)hydantoin and 160 ml. of concentrated ammonium hydroxide is heated at 60° for 4 days in a 200-ml. pressure bottle. The solution is evaporated to dryness under reduced pressure (Note 16).

(e) *Lysine-1-C^{14} Dihydrochloride.* The crude residue from the amination reaction is hydrolyzed by heating with 167 ml. of aqueous barium hydroxide solution (83 mmoles) in an autoclave at 160° for 30 minutes. The filtered solution is treated with an excess of ammonium carbonate (10 g.), filtered again and evaporated to dryness under vacuum. The residue is acidified with dilute hydrochloric acid and evaporated to dryness. The salt mixture is dissolved in a boiling solution of 7.7 g. of picric acid and 400 ml. of water and stored in a refrigerator for 2 days. The yield of crystalline dipicrate is 68.5%; m.p. 188°, after recrystallization from water (Note 17). The dihydrochloride is obtained by treating the dipicrate with an excess of dilute hydrochloric acid, extracting the picric acid with ether, decolorizing with charcoal and evaporating to dryness under vacuum. The crude product is triturated with absolute alcohol, collected on a filter and dried at room temperature (Note 18).

B. Notes

1. The procedure is an adaptation of Adamson's[2] synthesis of α-amino acids, which employs the Schmidt reaction[5] and α-aminodicarboxylic acids.

2. This synthesis is by the general method of Levine and Hauser[1] for preparing β-keto esters.

3. The sodamide is prepared in a 100-ml. three-necked round-bottomed flask equipped with a mercury-sealed stirrer, separatory funnel and reflux condenser. To the mixture of sodium and liquid ammonia is added a crystal of ferric nitrate catalyst and stirring is continued until the disappearance of blue color. An excess of sodamide is highly desirable.

4. Carbon dioxide is recovered by bubbling through a chain of absorbers containing *N* sodium hydroxide and saturated aqueous barium hydroxide.

5. The combined recovery of barium carbonate-C^{14} is 4.52 g. (25% on a radiochemical basis).

6. Excess acid gives an acid reaction to litmus and destroys the characteristic yellow color of diazomethane.

7. Levine and Hauser[1] have reported a 38% yield of methyl 2-oxocyclohexanecarboxylate, b.p. 94–95° (10 mm.), based on cyclohexanone and carbonation with Dry Ice.

8. The chloroform solution of hydrazoic acid,[5] prepared from sodium azide and sulfuric acid, is dried for a short time over sodium sulfate.

9. Adamson found this product to be 2-aminoheptanedioic acid hydrochloride mixed with some lysine dihydrochloride. In the absence of hydrolysis of the intermediate lactam, a second molecule of hydrazoic acid does not yield lysine.

10. For conversion to the monohydrochloride, see lysine-6-C^{14}.

11. Directions are given by Maimind for preparing the starting material by oxidation of 5.0 g. of N^6-benzoyllysine with sodium hypochlorite; yield 6.2–7.2 g. (purity 57–58%). The 2,4-dinitrophenylhydrazone melts at 163.5–164.5°. Also see 3-(3,4-dihydroxyphenyl)alanine-1-C^{14}.

12. See lysine-6-C^{14} monohydrochloride, Method I.

13. The method is the Bucherer modification of the Strecker synthesis of α-amino acids.[4] The yields and physical constants of intermediates are taken from the large-scale nonisotopic preparation.

14. A mixture of 10 g. (0.119 mole) of dihydropyran and 40 ml. of 0.2 *N* hydrochloric acid is refluxed for 15–20 minutes.[6] The stirred homogeneous solution is cooled quickly, neutralized with sodium hydroxide and treated with 11.4 g. of sodium pyrosulfite. An aliquot is used.

15. The yield is 73.3% based on 1.19 moles of dihydropyran (1.2 moles of cyanide).

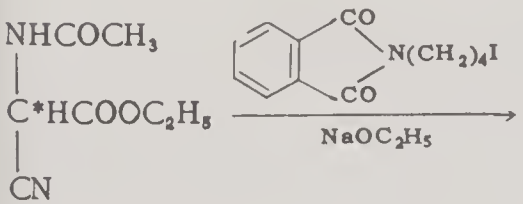
16. The product may be isolated as the hydrochloride (difficult to crystallize) by boiling an aqueous solution (100 ml.) of the residue with 4 g. of freshly prepared silver oxide, acidifying the filtered solution with hydrochloric acid and evaporating. The picrate monohydrate is obtained by treating an aqueous solution of the hydrochloride with one equivalent of picric acid; the m.p. is 180–183° after recrystallization from water.

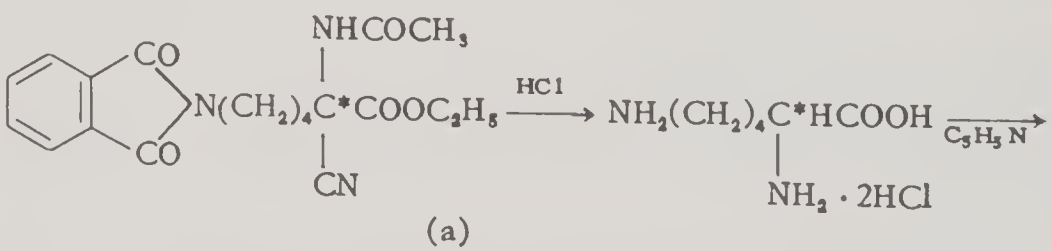
17. The isotopic dipicrate is converted to the monopicrate (no directions given) and resolved[7] enzymatically.

18. The salt is the dihydrate,[3] m.p. 186–189°; yield 88% based on the picrate. Borsook[7] has reported the quantitative conversion of the picrate to the anhydrous dihydrochloride.

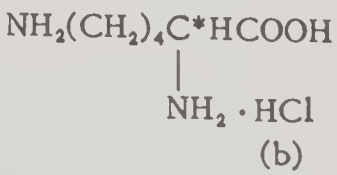
[1]R. Levine and C. R. Hauser, J. Am. Chem. Soc., *66*, 1768 (1944).
[2]D. W. Adamson, J. Chem. Soc., *1939*, 1564.
[3]R. Gaudry, Can. J. Research, *26B*, 387 (1948).
[4]H. T. Bucherer and V. A. Lieb, J. prakt. Chem., *141*, 5 (1934); Chem. Abstracts, *29*, 127 (1935).
[5]*Organic Reactions*, Vol. III, Wiley, New York, 1946, p. 307.
[6]L. E. Schniepp and H. H. Geller, J. Am. Chem. Soc., *68*, 1646 (1946).
[7]H. Borsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighley and P. H. Lowy, J. Biol. Chem., *176*, 1383 (1948).

LYSINE-2-C^{14} MONOHYDROCHLORIDE





(a)



(b)

M. Fields, D. E. Walz and S. Rothchild, J. Am. Chem. Chem. Soc., 73, 1000 (1951).

A. Procedure (Note 1)

(a) *Ethyl 2-Acetamido-2-cyano-6-phthalimidohexanoate-2-C^{14}.* To a boiling solution of 0.851 g. of ethyl acetamidocyanoacetate-2-C^{14} in 5 ml. of absolute alcohol, in a 100-ml. round-bottomed, three-necked flask equipped with a mercury-sealed stirrer, reflux condenser, and steam-jacketed, pressure-equalizing dropping funnel, is added a solution of 0.115 g. of sodium in 7 ml. of ethanol, followed by 1.68 g. of *N*-(4-iodobutyl)phthalimide[1,2] dissolved in 10 ml. of boiling ethanol. The mixture is refluxed overnight, then cooled and diluted with 15 ml. of water, and the product is collected; yield 1.86 g. (80%), m.p. 170–171.5° (Note 2).

(b) *Lysine-2-C^{14} Monohydrochloride.* A mixture of 1.53 g. of crude ethyl 2-acetamido-2-cyano-6-phthalimidohexanoate-2-C^{14} and 10 ml. of concentrated hydrochloric acid is refluxed overnight. The mixture is diluted with 10 ml. of water, the phthalic acid is filtered off, and the filtrate is evaporated under vacuum. The residual lysine-2-C^{14} dihydrochloride is dissolved in 10 ml. of hot alcohol and centrifuged to remove ammonium chloride. The hot supernatant solution is treated with 0.400 g. of pyridine to yield the crude product monohydrochloride. Recrystallization from 1 ml. of water, to which 9 ml. of ethanol is added, yields 0.563 g. of pure product, m.p. 259–262° (dec.) (evacuated tube). The filtrate yields an additional 0.094 g. of pure product, making the yield 87% (Note 3).

B. Notes

1. This method of synthesizing α-amino acids is general.

2. The pure compound, crystallized from alcohol, melts at 172–172.5°. Use of the bromo starting material decreases the yield to 73%. The iodo starting material may be prepared almost quantitatively from the bromo or chloro compound by heating overnight in acetone solution with sodium iodide.

3. Lysine-2-C^{14} dipicrate,[2,3] m.p. 186–188°, is prepared from the hydrochloride in aqueous solution.

Cadaverine-1,5-$C^{14}_{1/2}$, (1,5-pentanediamine-1,5-$C^{14}_{1/2}$), is prepared[4] by bacterial decarboxylation of lysine-2-C^{14}, and purified as the dipicrate, m.p. 221°.

C. Other Preparations

The following two compounds are prepared from *N*-(3-iodopropyl)phthalimide by the same procedure:

Ethyl 2-acetamido-2-cyano-5-phthalimidovalerate-2-C^{14}; yield 75%, m.p. 211–213°. The recrystallized material melts at 214.5–215°. Use of the bromo starting material decreases the yield to 54%.

Ornithine-2-C^{14} monohydrochloride, (2,5-diaminovaleric-2-C^{14} acid monohydrochloride); yield 79%, m.p. 215–216° (evacuated tube). The dipicrate melts at 196–197°.

Lysine-2-C^{14} has been prepared by Arnstein[2] by the quite similar, but older, Sörensen phthalimidomalonic ester method. 4-Iodobutylphthalimide was condensed with ethyl phthalimidomalonate-2-C^{14}, and the condensation product was hydrolyzed in acid solution. The yield was 18.9% based on ethyl malonate-2-C^{14}. By the use of carriers, the racemic mixture was partially resolved into D-lysine-2-C^{14} (80%) and L-lysine-2-C^{14} (88–90% resolution) through the intermediate dipicrates.

Lysine-2-C^{14} dipicrate has been prepared by Fields[5] in 45% yield. To a chloroform solution of 2-carboxyheptanedioic-2-C^{14} acid and 100% sulfuric acid at 60° was added a small excess of hydrazoic acid in chloroform, forming lysine-2-C^{14}. The yield in the Schmidt reaction[6] showed marked temperature dependence.

[1]S. Gabriel, Ber., *42*, 1249 (1909).

[2]H. R. V. Arnstein, G. D. Hunter, H. M. Muir and A. Neuberger, J. Chem. Soc., *1952*, 1329.

[3]R. Gaudry, Can. J. Research, *26B*, 387 (1948).

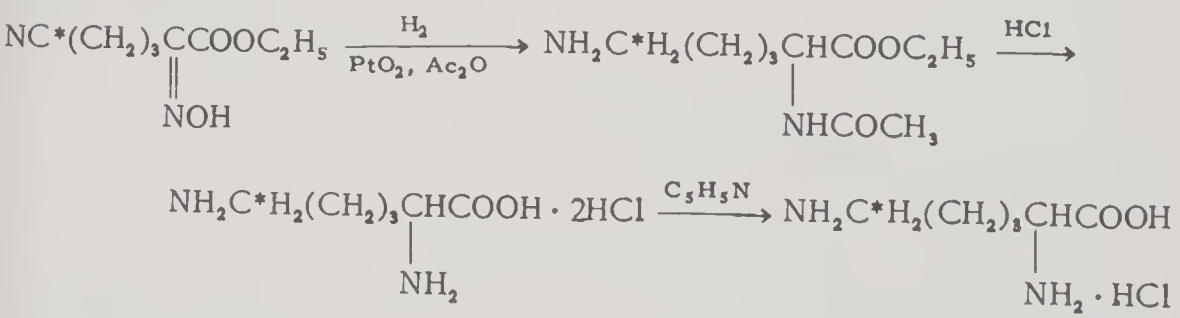
[4]R. W. Schayer, R. L. Smiley and J. Kennedy, J. Biol. Chem., *206*, 461 (1954); E. F. Gale, Biochem. J., *34*, 392 (1940).

[5]M. Fields, S. Rothchild, J. Gibbs and D. Walz, Nucleonics, 7 (3), 63 (1950).

[6]*Organic Reactions*, Vol. III, Wiley, New York, 1946, Chapt. 8.

LYSINE-6-C^{14} MONOHYDROCHLORIDE

METHOD I



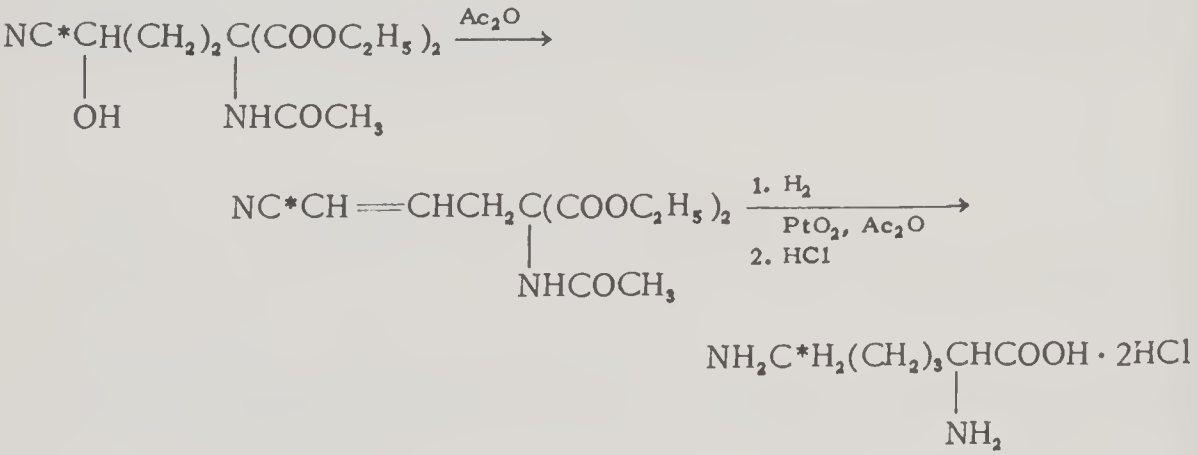
P. Olynyk, D. B. Camp, A. M. Griffith, S. Woislowski, and R. H. Helmkamp, J. Org. Chem., *13*, 465 (1948).

A. Procedure (Note 1)

A mixture of 11.1 g. of ethyl 5-cyano-C^{14}-2-hydroxyiminovalerate, 60 ml. of acetic anhydride and 0.3 g. of Adams catalyst is shaken at 50° with hydrogen at a pressure of 50–60 p.s.i. Fresh catalyst is added at

65% of the theoretical hydrogen uptake, and the temperature is raised to 60°. At 85% of complete reaction, 20 ml. of acetic anhydride and another portion of catalyst are added, the temperature is raised to 65°, and the hydrogenation is continued to completion (Note 2). The solvent is decomposed with cold water, and the catalyst is filtered out. The ethyl 2-acetamido-6-aminohexanoate-6-C^{14} is hydrolyzed by refluxing the filtrate overnight with 1.5 volumes of concentrated hydrochloric acid. The mixture is concentrated under reduced pressure on a steam-bath, a small amount of concentrated hydrochloric acid is added to the syrupy residue and, after evaporation, the step is repeated again. The residue is dissolved in hot absolute alcohol, and the cooled solution is diluted slowly with ether to precipitate lysine-6-C^{14} dihydrochloride, which is collected on a filter (Note 3). To a hot stirred solution of the dihydrochloride in 95% alcohol is added a 28% excess of pyridine in hot alcoholic solution. After cooling overnight in a refrigerator, the product is collected, washed with cold absolute ethanol and dried; yield 8.0 g. (73%), m.p. 264–266° (dec.) (Note 4).

METHOD II



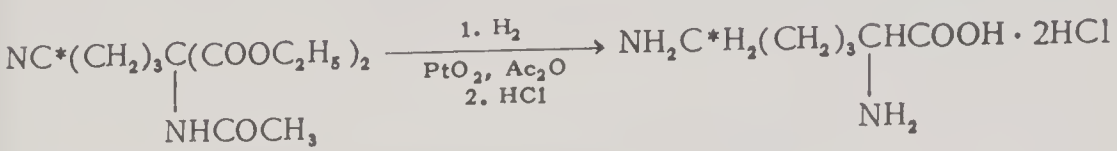
S. Linstedt, Acta Chem. Scand., 7, 340 (1953).

A. Procedure

A mixture of 0.500 g. of ethyl 2-acetamido-2-carbethoxy-5-cyano-C^{14}-5-hydroxyvalerate and 5 ml. of acetic anhydride is refluxed for 1.5 hours. The mixture is then hydrogenated at room temperature over Adams catalyst at 100 atmospheres pressure for 24 hours. The mixture is filtered, and the oil, resulting from evaporation of the solvent under vacuum, is hydrolyzed by refluxing with 25 ml. of 6 *N* hydrochloric acid for 15 hours. The crude product is purified by chromatography as described for 5-hydroxylysine-6-C^{14}. The yield of lysine-6-C^{14} dihydro-

chloride is 0.130 g. (36%). The conversion to the monohydrochloride may be made in the manner described above.

METHOD III



M. Rothstein and C. J. Claus, J. Am. Chem. Soc., 75, 2981 (1953).

A. Procedure

Crude ethyl 2-acetamido-2-carbethoxy-5-cyano-C^{14}-valerate (22 mmoles) is hydrogenated in acetic anhydride solution (60 ml.) over Adams catalyst (0.3 g.) at 60 p.s.i. and 50° for 12 hours (Note 5). Another portion of catalyst (0.2 g.) is added, and the reaction is continued for an additional 24 hours. The acetic anhydride is destroyed by the addition of ice water with standing for 1 hour. The catalyst is filtered off, and the solution is refluxed for 20 hours with 1.5 volumes of concentrated hydrochloric acid. The dihydrochloride is isolated and converted to the monohydrochloride in the manner described above; yield 1.90 g. (Note 6).

B. Notes

1. The procedure is a modification of the Fischer-Weigert[1] synthesis of lysine and is practically the same as the isotopic synthesis developed by Borsook.[2] The isolation and purification are by the procedure of Eck and Marvel.[3]

2. It is important that the theoretical amount of hydrogen be absorbed; 3 to 5 days are required.

3. Pure lysine dihydrochloride melts at 187–189°.

4. The recovery of monohydrochloride from the dihydrochloride is of the order of 91–94%.

5. The oily starting material cannot be crystallized.

6. Four trial runs gave over-all yields of 27–29%, and another gave 39% based on potassium cyanide. The yield in the isotopic preparation is 21%, or 38% based on the cyanide-C^{14} not recovered. No 2-aminohexanedioic acid is detected in the product by two-dimensional paper chromatography.

C. Other Preparations

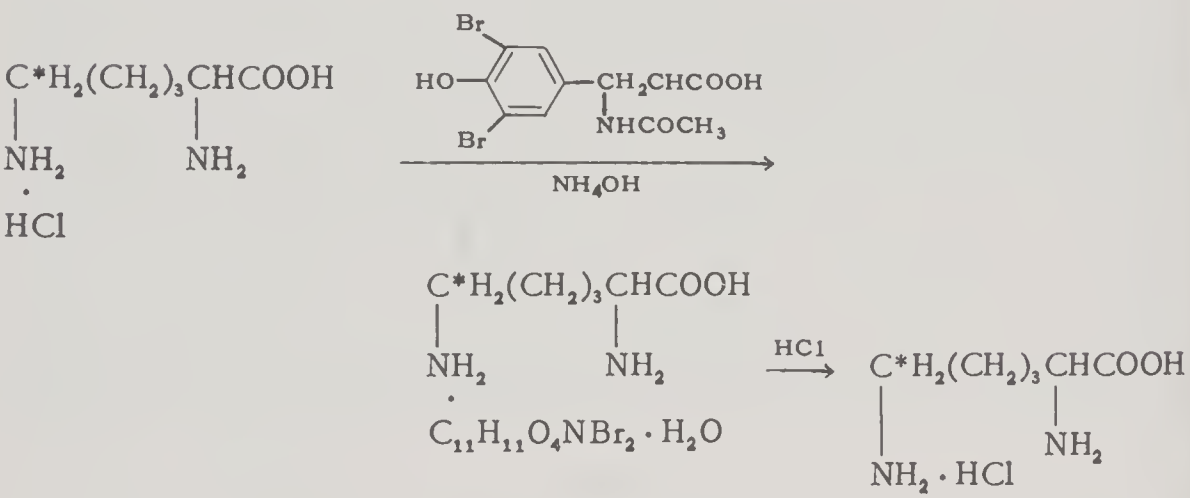
Lysine-6-C^{14} dihydrochloride has been prepared by Borsook[2] (26% yield), by reducing ethyl 5-cyano-C^{14}-2-hydroxyiminovalerate with sodium and alcohol, followed by acid hydrolysis. The compound is resolved by

the carbobenzyloxy-aniline-papain method[2] of Bergmann; see L-lysine-6-C^{14} dihydrochloride.

[1]E. Fischer and F. Weigert, Ber., *35*, 3772 (1902).
[2]H. Borsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighly and P. H. Lowy, J. Biol. Chem., *176*, 1383 (1948); *ibid.*, *173*, 423 (1948).
[3]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 374.

L-LYSINE-6-C^{14} MONOHYDROCHLORIDE

METHOD I



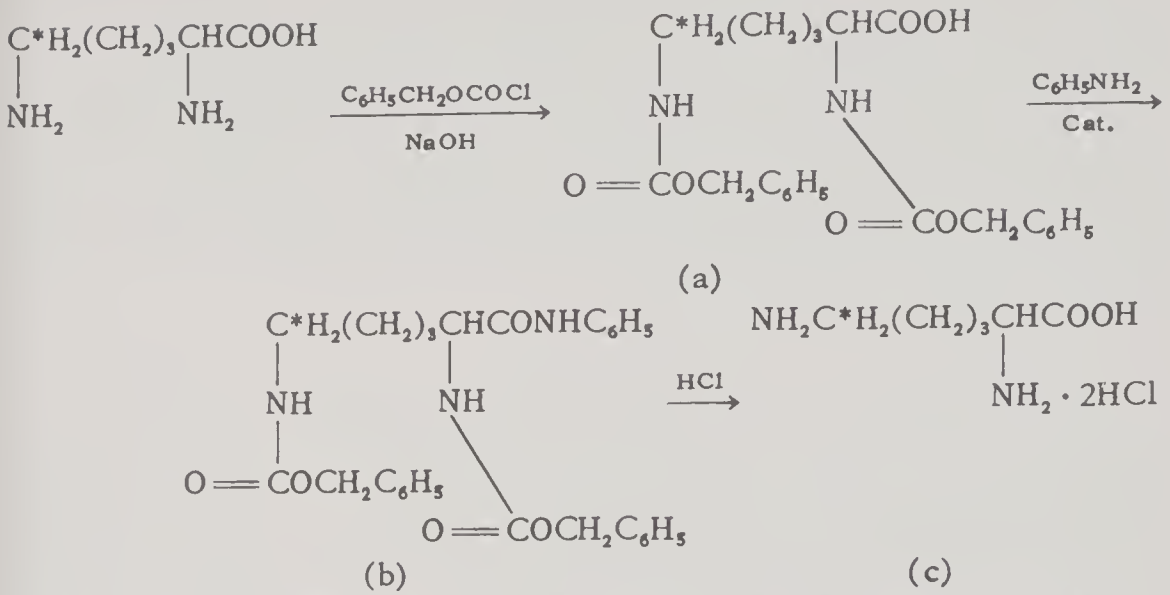
M. Rothstein and L. L. Miller, J. Biol. Chem., *206*, 243 (1954).

A. Procedure (Note 1)

To a hot stirred solution of 2.00 g. of *N*-acetyl-3,5-dibromo-L-tyrosine (Note 2) in 5 ml. of water, 5 ml. of methanol and 0.32 ml. of concentrated ammonium hydroxide is slowly added 1.87 g. of DL-lysine-6-C^{14} monohydrochloride, and the mixture is stored in a refrigerator for 4 hours. The crystalline *N*-acetyl-3,5-dibromo-L-tyrosine L-lysine-6-C^{14} salt is collected and washed with cold methanol (Note 3).

The salt is dissolved in water, and the solution is acidified with concentrated hydrochloric acid (total volume 40 ml.) and stored in a refrigerator for 2 days. The filtered solution is concentrated to 5 ml. under reduced pressure and again filtered to remove a small amount of acetyldibromotyrosine. The filtrate is evaporated to dryness under vacuum, and the residue is converted to L-lysine-6-C^{14} monohydrochloride in the manner described for the DL-compound, Method I; yield 0.564 g., 60%. Purification is accomplished by reprecipitation from water with alcohol; $[\alpha]_D^{25}$ + 18.8° (*c* 1 in 0.6 *N* hydrochloric acid).

METHOD II



H. Borsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighley and P. H. Lowy, J. Biol. Chem., *176*, 1383 (1948).

A. Procedure (Note 4)

(a) N-*Carbobenzyloxy-DL-lysine-6-C*^{14}. To an ice-cooled solution of 0.430 g. of DL-lysine-6-C^{14} dihydrochloride in 2.9 ml. of 2 *N* sodium hydroxide is added 1.1 ml. of benzyl chloroformate[1] and 2.3 ml. of 4 *N* sodium hydroxide in four portions. The mixture is shaken vigorously for 25 minutes with icecooling, then washed with ether. The aqueous solution is acidified with hydrochloric acid, and the product is isolated by ether extraction and evaporation under vacuum; yield 0.733 g., m.p. 100–103°.

(b) N-*Carbobenzyloxy-L-lysine-6-C*^{14} *Anilide*. To a solution of 0.730 g. of *N*-carbobenzyloxy-DL-lysine-6-C^{14} in 1.9 ml. of *N* sodium hydroxide and 1.8 ml. of water are added 0.46 ml. of aniline, 7.1 ml. of 0.3% aqueous cysteine hydrochloride solution, 8.2 ml. of citrate buffer solution (pH 5.0), 16.4 ml. of water and 2.5 ml. of a solution containing 0.100 g. of papain, 2 ml. of water and 2 ml. of citrate buffer. The mixture is stored at 40° for 19 hours. The product is filtered off and washed with 1% potassium bicarbonate solution, followed by three portions of water; yield 0.465 g. (Note 5).

(c) *L-Lysine-6-C*^{14} *Dihydrochloride*. The *N*-carbobenzyloxy-L-lysine-6-C^{14} anilide is refluxed for 2 hours with 4.5 ml. of 6 *N* hydrochloric acid. The cooled mixture is diluted with 30 ml. of water and made alkaline with freshly prepared silver oxide. After the silver compounds are filtered off and washed with water, the filtrate is washed with ether. The clear aqueous solution is concentrated to 20 ml., acidified with hydrochloric acid and set aside for 30 minutes. The solution is filtered and evaporated to dryness under vacuum over sulfuric acid and sodium hydroxide; yield quantitative (Note 6).

(d) *D-Lysine-6-C*^{14} *Dihydrochloride.* The mother liquor from the *N*-carbobenzyloxy-L-lysine-6-C^{14} anilide preparation (without washings) is treated with 0.025 g. of papain at 40° for 5 days in the manner described. The mixture is filtered, and the filtrate is acidified with hydrochloric acid and extracted with ether. Evaporation yields 0.243 g. of *N*-carbobenzyloxy-D-lysine-6-C^{14}. After refluxing with 3 ml. of 6 *N* hydrochloric acid for 2 hours, extraction with ether and evaporation of the aqueous phase, 0.130 of crude product is obtained (Note 7).

B. Notes

1. The procedure is an adaptation of that of Kearley.[2]

2. Directions are given by DeWitt[3] for preparing *N*-acetyl-3,5-dibromo-L-tyrosine, m.p. 213–215° (dec.) from L-tyrosine.

3. The filtrate is used successively to complete the transfer (then is saved for racemization of the D-lysine-6-C^{14} and unchanged L-lysine-6-C^{14}). The pure salt monohydrate, recrystallized from 1.5 parts of water and an equal volume of methanol, melts at 166–168° (dec.); yield 84%, $[\alpha]_D^{25}$ + 36.8 ± 0.3° (*c* 4, water).[2]

4. The resolution is by the carbobenzyloxy-aniline papain method of Bergmann;[4] see L-leucine-1-C^{14}

5. The melting point is 121–122° after recrystallization from 50% ethanol.

6. $[\alpha]_D^{23}$ + 15.95°, in water, *c* = 5.46.

7. Recrystallization from alcohol gives 0.099 g. of material exhibiting a rotation corresponding to approximately 92.5% D- and 7.5% L-form; $[\alpha]_D^{23}$ −13.5° in water, *c* = 4.47.

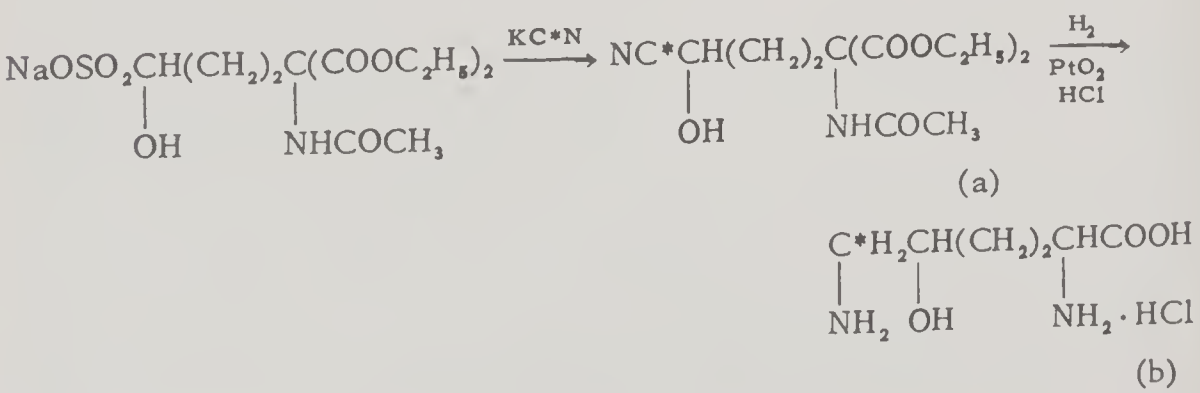
[1] *Organic Syntheses,* Vol. 23, Wiley, New York, 1943, p. 13.

[2] F. J. Kearley and A. W. Ingersoll, J. Am. Chem. Soc., *73,* 5783 (1951).

[3] H. D. Dewitt and A. W. Ingersoll, J. Am. Chem. Soc., *73,* 5782 (1951).

[4] M. Bergmann and H. Fraenkel-Conrat, J. Biol. Chem., *119,* 707 (1937); J. S. Fruton, G. W. Irving, Jr., and M. Bergmann, *ibid., 133,* 703 (1940); O. K. Behrens, D. G. Doherty and M. Bergmann, *ibid., 136,* 61 (1940).

5-HYDROXYLYSINE-6-C^{14} MONOHYDROCHLORIDE



Sven Lindstedt, Acta Chem. Scand., 7, 340 (1953).

A. Procedure (Note 1)

(a) *Ethyl 2-Acetamido-2-carbethoxy-5-cyano-C^{14}-5-hydroxyvalerate, (4-Acetamido-4,4-dicarbethoxybutyraldehyde Cyanohydrin-C^{14}).* To 5 ml. of a solution of the sodium bisulfite complex of 4-acetamido-4,4-dicarbethoxybutyraldehyde (Note 2) is added 0.210 g. of potassium cyanide-C^{14} in 2 ml. of water. After being shaken for 30 minutes, the product is extracted into ethyl acetate solution, which is then dried over sodium sulfate and evaporated to dryness. Addition of a little ether gives 0.840 g. (86.7%) of crystalline product. After one recrystallization from benzene the compound melts at 82° (Note 3).

(b) *5-Hydroxylysine-6-C^{14} Monohydrochloride.* 4-Acetamido-4,4-dicarbethoxybutyraldehyde cyanohydrin-C^{14} (0.650 g.), in 20 ml. of glacial acetic acid-ethanol (1:9), is hydrogenated over 0.1 g. of Adams catalyst at room temperature and atmospheric pressure (Note 4). The reduction is complete in about 6 hours. The mixture is filtered and the solvents are removed under vacuum. The residual oil is hydrolyzed by refluxing with 25 ml. of 6 *N* hydrochloric acid for 15 hours. The mineral acid is removed by repeated evaporation under vacuum, and the residue is dissolved in 100 ml. of water and passed through an ion exchange column (Note 5) to remove small amounts of lysine-6-C^{14}. The column, washed chloride-free with water, then is fractionally eluted with 0.15 *N* ammonium hydroxide in 4-ml. portions. The fractions containing pure product, as determined by paper chromatography, are combined and evaporated to dryness. The residual 5-hydroxylysine-6-C^{14} dihydrochloride is dissolved in a minimal amount of 50% alcohol; then the solution is treated with 0.5 ml. of pyridine, and the product monohydrochloride is crystallized by addition of a little acetone; yield 0.207 g., 51% (Note 6).

B. Notes

1. This preparation is by a modification of the procedure of Touster.[1]

2. To 21.7 g. of ethyl acetamidomalonate and 2 ml. of 0.5 *N* sodium ethoxide in 35 ml. of benzene is added 7 ml. of acrolein with stirring. Two hours later, a solution of 12.6 g. of sodium bisulfite heptahydrate in 35 ml. of water and 5.8 ml. of glacial acetic acid is added. After being stirred overnight, the solution is filtered and diluted to 100 ml. with water. This preparation, by the 1,4-addition of a malonate and an α, β-unsaturated aldehyde, was developed by Moe and Warner.[2]

3. An analytically pure sample melts at 86.5–87.5°.[3]

4. Hydrogenation in acetic acid-acetic anhydride solution at 3 atmospheres pressure forms considerable lysine.[1]

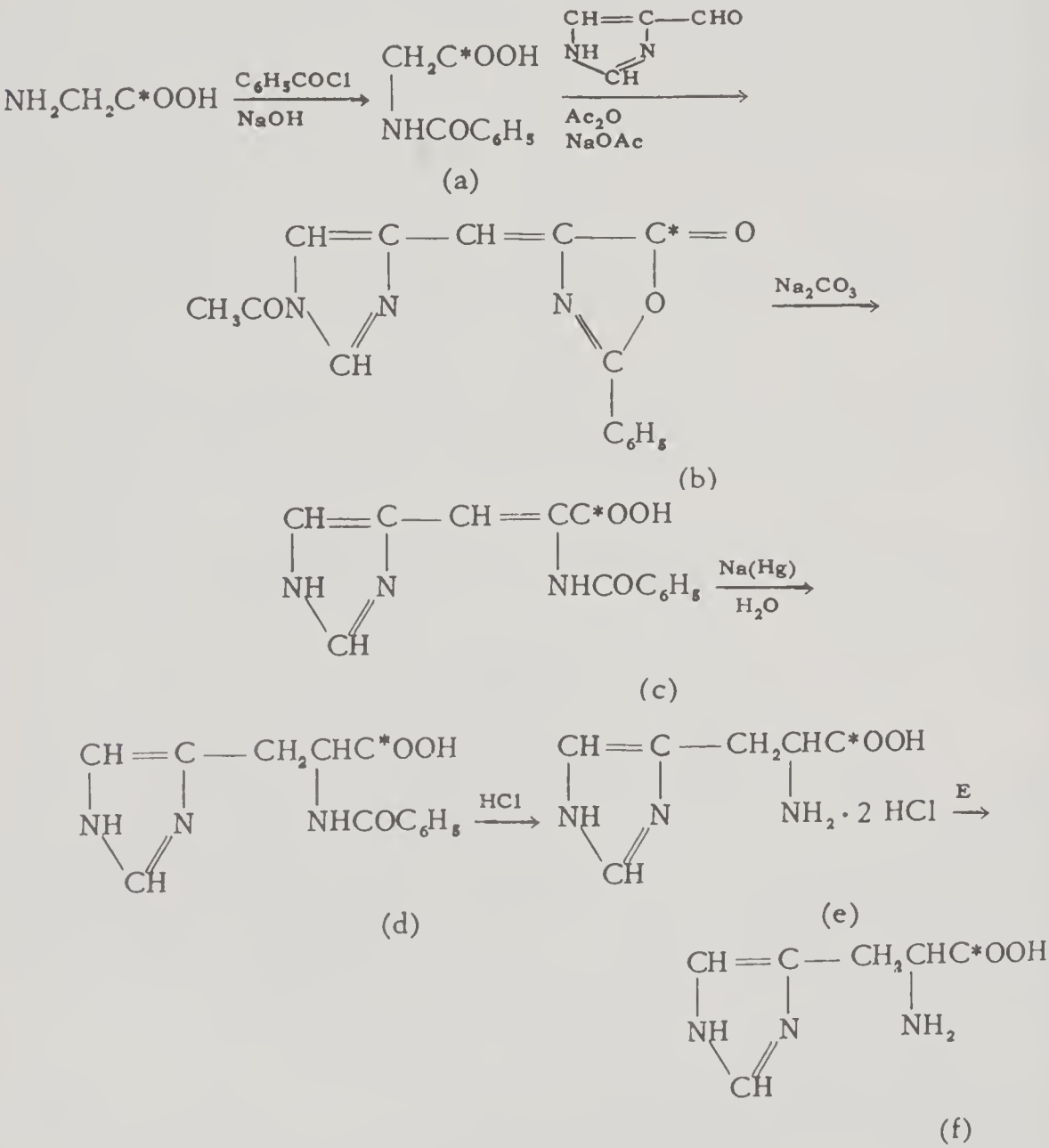
5. A 35-sq. mm. × 350-mm. column of 100–200 mesh Dowex-50, in the ammonium form, is used as recommended by Weissiger.[4]

6. The compound is assayed by periodic acid oxidation,[1] yielding ammonia, and formaldehyde which is weighed as the dimedon derivative; the equivalent weight is 198 (calculated 198.66).

[1]O. Touster, J. Am. Chem. Soc., *73*, 491 (1951).
[2]O. A. Moe and D. T. Warner, *ibid.*, *70*, 2763 (1948).
[3]D. T. Warner and O. A. Moe, *ibid.*, *70*, 3918 (1948).
[4]J. R. Weissiger, J. Biol. Chem., *186*, 591 (1950).

α-AMINO-4(or 5)-IMIDAZOLEPROPIONIC-C^{14} ACID (C_1^{14}-Histidine)

METHOD I



H. Borsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighley and P. H. Lowy, J. Biol. Chem., *196*, 669 (1952).

A. Procedure (Note 1)

(a) *Hippuric-C^{14} Acid*, (*N-Benzoylglycine-1-C^{14}*). To a stirred solution of 2.11 g. of glycine-1-C^{14} in 22 ml. of 10% sodium hydroxide is added gradually a mixture of 9.65 ml. of benzoyl chloride and 45 ml. of 10% sodium hydroxide. The mixture is stirred until the excess benzoyl chloride is hydrolyzed; then the product is precipitated by acidification with hydrochloric acid. Purification is accomplished by repeated extraction of the benzoic acid with boiling ligroin; yield 4.96 g. (98.5%), m.p. 183–185°.

(b) *4-(1-Acetyl-4-imidazolylmethylene)-2-phenyl-2-oxazolin-5-one-5-C^{14}* (Note 2). An intimate mixture of 4.96 g. of hippuric-C^{14} acid, 3.10 g. of 4(or 5)-imidazolecarboxaldehyde (Note 3), 3.10 g. of fused sodium acetate and 12 ml. of acetic anhydride is heated on a water-bath for 30 minutes, then cooled, and the yellow needles are collected. The product is triturated with cold water, washed with water and alcohol, and air-dried; yield 5.74 g., 74% (Note 4).

(c) *α-Benzamido-4(or 5)-imidazoleacrylic-C^{14} Acid.* The oxazolinone (11.95 g.) is hydrolyzed by the method of Pyman,[1] which follows. The yield of monohydrated acid is 9.81 g. (84%).

4-(1-Acetyl-4-imidazolylmethylene)-2-phenyl-2-oxazolin-5-one (5 g.) is dissolved in a solution of 2.5 g. of anhydrous sodium carbonate in 125 ml. of water by boiling (15 minutes). The solution is decolorized with charcoal and treated with 1.76 g. of glacial acetic acid. The crystalline product is collected, washed and air-dried; yield 88%, m.p. 208° (cor.) (dec.).

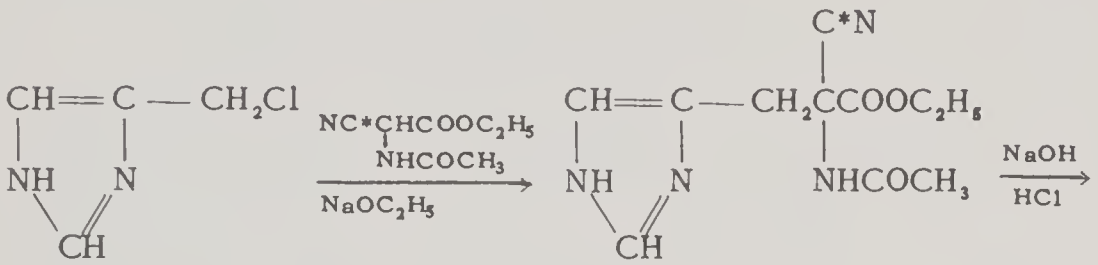
(d) *α-Benzamido-4(or 5)-imidazolepropionic-C^{14} Acid*, (*C_1^{14}-N-Benzoylhistidine*). To a stirred mixture of 1.50 g. of α-benzamido-4(or 5)-imidazoleacrylic-C^{14} acid and 15 ml. of water is added gradually 20 g. of 2.15% sodium amalgam. The aqueous solution is separated from the mercury and acidified to pH 5.2 with hydrochloric acid. The product is collected on a filter, washed with water and combined with an additional crop obtained by concentrating the mother liquors; yield 51%, m.p. 238–239° (Note 5).

(e) *α-Amino-4(or 5)-imidazolepropionic-C^{14} Acid Dihydrochloride.* A mixture of 1.95 g. of C_1^{14}-*N*-benzoylhistidine and 100 ml. of 20% hydrochloric acid is refluxed for 4 hours. The mixture is extracted with ether, to remove benzoic acid, then evaporated to dryness under reduced pressure (Note 6).

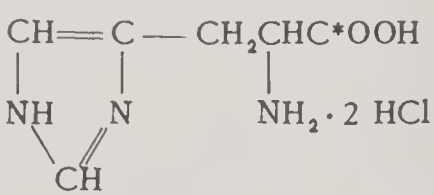
(f) *α-Amino-4(or 5)-imidazolepropionic-C^{14} Acid* (Note 7). A solution of 0.170 g. of the dihydrochloride in 100 ml. of water is placed in a glass cell, in which are suspended two cellophane bags (2.5 cm. wide), each containing water to the level of the surrounding solution, and a platinum wire mesh electrode. The electrodes are connected in series

with a 30-watt, 115-volt lamp and to a source of 120-volt direct current. The solution is stirred occasionally, and every 30 minutes the electrodialysis is interrupted and the catholyte and anolyte are replaced by distilled water. The combined catholytes, collected during 4 hours, are evaporated to dryness under vacuum; yield 97% (Note 8).

METHOD II



(a)



(b)

L. P. Bouthillier and A. D'Iorio, Rev. can. biol., 9, 382 (1950).

A. Procedure (Note 9)

(a) *Ethyl α-Acetamido-α-cyano-C*^{14}*-4(or 5)-imidazolepropionate.* To a cold solution of 0.52 g. of sodium in 50 ml. of absolute ethanol is added 2 g. of ethyl acetamidocyano-C^{14}-acetate. The mixture is stored for 1 hour in a refrigerator; then 1.8 g. of 4(or 5)-(chloromethyl)imidazole hydrochloride (Note 10) is added to the cold solution, and the mixture is stirred at room temperature for 1 hour. The salt is filtered off, and the filtrate is concentrated under reduced pressure. The crystalline product monohydrate is obtained by triturating the residual syrup with 1 ml. of water.

(b) *α-Amino-4(or 5)-imidazolepropionic-C*^{14} *Acid Dihydrochloride.* The crude condensation product, in a small flask equipped with a side tube closed by a stopcock, is treated with an amount of 0.1 *N* sodium hydroxide calculated to saponify the carbethoxy group. The mixture is refluxed for 1 hour, then concentrated under vacuum to several ml. The reflux condenser is attached to a gas washing bottle containing 20% sodium hydroxide solution for trapping the liberated carbon-C^{14} dioxide. The mixture is acidified with 30 ml. of concentrated hydrochloric acid

and refluxed for 5 hours in a gentle stream of nitrogen, admitted through the side tube. The hydrolysate is evaporated to dryness under reduced pressure. The residue is taken up in 5 ml. of hot concentrated hydrochloric acid, and the sodium chloride is filtered off on a sintered-glass funnel and washed with three 2-ml. portions of concentrated acid. The combined filtrate is evaporated to dryness under reduced pressure, and the residue is taken up in 4 ml. of concentrated hydrochloric acid. The filtered solution is evaporated to 2 ml. in an air stream, then diluted with 4 ml. of absolute ethanolic hydrogen chloride solution and seeded, and the product is collected and washed with several ml. of the alcoholic solution. The yield of product, m.p. 220°, is 6% based on sodium cyanide; the molar specific activity is 81% of that of the starting material.

B. Notes

1. The procedure is adapted from the method of Pyman;[1] see the review on azlactones by Carter.[2]

2. The procedure is a modification of the method of Ingersoll and Babcock.[3]

3. The starting material is prepared by the oxidation[1] of 4(or 5)-(hydroxymethyl)imidazole hydrochloride[4] with the requisite amount of concentrated nitric acid.

4. The product crystallizes from chloroform in bright yellow needles, m.p. 191° (cor.). It slowly dissolves in hot alcohol with decomposition.

5. The compound crystallizes from water with one molecule of water which is lost at 115°, but not at 100°.

6. Dissolution in water and treatment with hydrogen chloride in alcohol deposits the crude dihydrochloride, m.p. 210°; purified m.p. 235° (cor.) (dec.). Purification[1] is accomplished through formation of the dipicrate, m.p. 100–103°.

7. The usual isolation of the free base from a hydrochloride, by treatment with silver carbonate, gives a lower yield of a less pure product.

8. L-α-Amino-4(or 5)-imidazolepropionic-C^{14} acid L-tartrate is obtained in 83% yield from a solution of 0.821 g. of the C_1^{14}-DL-histidine and 0.800 g. of L-tartaric acid in 3.75 ml. of water by seeding and cooling. L-α-Amino-4(or 5)-imidazolepropionic-C^{14} acid is prepared from the tartrate by electrodialysis.

9. The procedure is a modification of the method of Albertson;[5] also see histidine-α-C^{14}.

10. The 4(or 5)-(chloromethyl)imidazole is prepared by chlorinating 4(or 5)-(hydroxymethyl)imidazole[4] with phosphorus pentachloride by the method of Pyman,[6] or with thionyl chloride by the method of Turner.[7]

C. Other Preparations

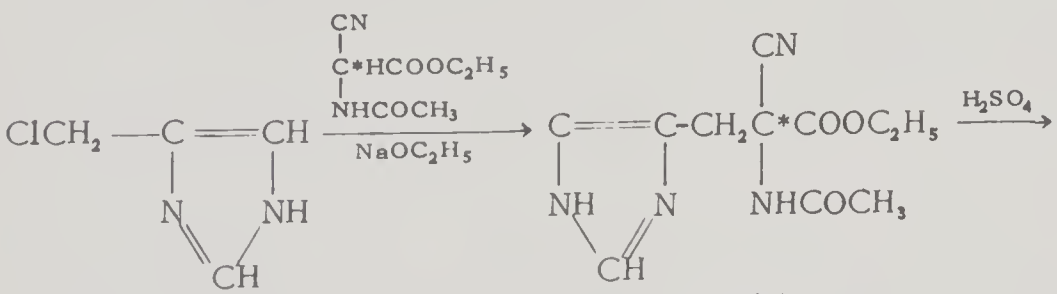
Hippuric-C^{13} acid has been prepared by Lerner,[8] and the preparation of hippuric-C^{14} acid (100% yield) by a similar procedure has been reported by Wang,[9] who employed ether for the extraction.

Hippuric-α-C^{14} acid has been prepared in like manner from glycine-2-C^{14}, by Adams[10] and Krasna,[11] while preparation of the -α-C^{13} acid has been reported by Lerner[8] (46% yield based on sodium acetate).

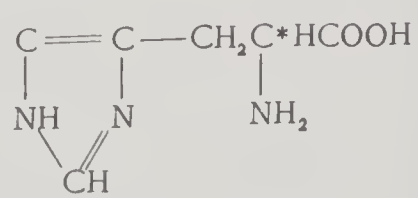
The preparation of hippuric-C^{14}-α-C^{14} acid from glycine-C_2^{14} has been reported by Schepartz[12] (56% yield based on ethyl bromoacetate), and Gurin.[13]

[1]F. L. Pyman, J. Chem. Soc., *109*, 186 (1916).
[2]*Organic Reactions*, Vol. III, Wiley, New York, 1946, p. 198.
[3]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 328.
[4]*Organic Syntheses*, Vol. 24, Wiley, New York, 1944, p. 64.
[5]N. F. Albertson and S. J. Archer, J. Am. Chem. Soc., 67, 308 (1945).
[6]F. L. Pyman, J. Chem. Soc., 99, 668 (1911).
[7]R. A. Turner, C. F. Huebner and C. R. Scholz, J. Am. Chem. Soc., *71*, 2801 (1949).
[8]A. B. Lerner, J. Biol. Chem., *181*, 281 (1949).
[9]S. C. Wang, J. P. Hummel and T. Winnick, J. Am. Chem. Soc., *74*, 2445 (1952).
[10]P. T. Adams and B. M. Tolbert, J. Am. Chem. Soc., *74*, 6272 (1952).
[11]A. I. Krasna, P. Peyser and D. B. Sprinson, J. Biol. Chem., *198*, 421 (1952).
[12]B. Schepartz and S. Gurin, J. Biol. Chem., *180*, 663 (1949).
[13]S. Gurin and A. M. Delluva, J. Biol. Chem., *170*, 545 (1947).

HISTIDINE-α-C^{14}
(α-Amino-4(or 5)-imidazolepropionic-α-C^{14} Acid)



(a)



(b)

George Wolf, Radiocarbon Laboratory, University of Illinois, Urbana, Illinois, private communication; J. Biol. Chem., *200*, 637 (1953).

A. Procedure (Note 1)

(a) *Ethyl α-Acetamido-α-cyano-4(or 5)-imidazolepropionate-α-C^{14}.* To a cold stirred suspension of 0.212 g. (1.244 mmoles) of ethyl acetamidocyanoacetate-2-C^{14} in 2.5 ml. of absolute ethanol (Note 2), in a 30-ml. test tube, is added 6.86 ml. of 0.3825 *N* sodium ethoxide (2.613 mmoles, Note 3) in absolute ethanol. The mixture is stirred for 30 minutes at 0°, then treated dropwise with a solution of 0.210 g. (1.37 mmoles) of 4(or 5)-(chloromethyl)imidazole hydrochloride in 2 ml. of absolute ethanol at 0° (Note 4). The mixture is stirred for 6 hours at room temperature; then the precipitated sodium chloride is separated and washed with two 1-ml. portions of ice-cold absolute ethanol. The combined solutions are evaporated at room temperature under reduced pressure, leaving the product as a colorless oil.

(b) *Histidine-α-C^{14}.* A mixture of the ester and 10 ml. of 15% sulfuric acid solution is refluxed for 12 hours. The cooled solution is added slowly to a mixture of 7 g. of barium hydroxide and 15 ml. of water. The suspension is stirred for 30 minutes; then the precipitate is filtered off and washed with several portions of water. The combined alkaline filtrate and washings are treated with solid ammonium carbonate (1 g.) until no more barium is precipitated. The barium carbonate is filtered off and washed with water. The combined filtrate and washings are boiled for 5 minutes, concentrated at 80° under reduced pressure and evaporated to dryness in an air stream at 80°. The oily residue, suspended in 3 ml. of boiling absolute ethanol, is mixed with sufficient water to effect solution, then is diluted with ethanol to the point of incipient turbidity. The mixture is cooled slowly to room temperature and stored overnight at 4°. The crystalline product is collected, washed with 1 ml. of cold 95% alcohol and dissolved in a minimum volume of water. The filtered solution is evaporated to dryness, and the residue is recrystallized in the manner described above; yield 0.128 g. (67.3%), m.p. 271–272°. The radiochemical yield is 65.5%. The molar specific activities of the product and the dimercury derivative are about 98% of that of the starting material. Paper chromatography and radioautography indicate the presence of only one compound.

B. Notes

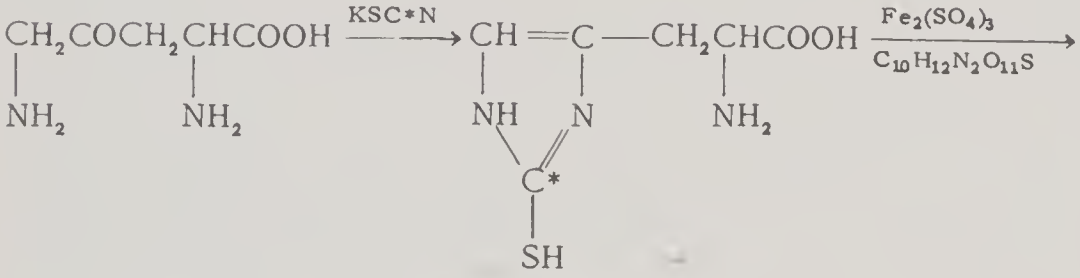
1. The method is adapted from that of Bouthillier and D'Iorio; see α-amino-4(or 5)-imidazolepropionic-C^{14} acid, Method II.

2. The fine suspension is obtained by rapid cooling of a warm solution with an ice-bath. The absolute alcohol is prepared by refluxing dry ethanol with aluminum ethoxide for 1 hour and distilling.

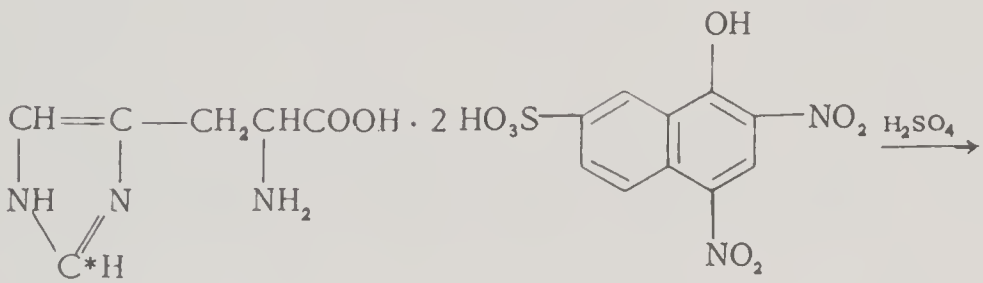
3. The alkali is standardized by titration with hydrochloric acid (phenolphthalein).

4. 4(or 5)-(Chloromethyl)imidazole hydrochloride is recrystallized several times from ethanol, m.p. 142°, and dried under vacuum over phosphorus pentoxide. The solution for use in obtained by cooling a warm solution to 0° for 10 minutes, such that the salt remains dissolved. Also see α-amino-4(or 5)-imidazolepropionic-C^{14} acid, Method II, Note 10.

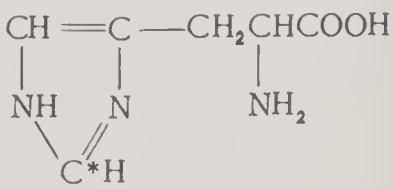
L-4(OR 5)-IMIDAZOLEALANINE-2-C^{14}
(L-Histidine-2-C^{14})



(a)



(b)



(c)

H. Borsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighley and P. H. Lowy, J. Biol. Chem., *187*, 839 (1950).

A. Procedure (Note 1)

(a) *L-2-Mercaptohistidine-2-C^{14}*. A mixture of sodium thiocyanate-C^{14} (prepared quantitatively from 0.056 g. of sodium cyanide-C^{14}), 0.296 g. of carrier sodium thiocyanate, 0.835 g. of 2,5-diaminolevulinic acid dihydrochloride (Note 2) and 2.0 ml. of water is heated on a steam-bath for 1 hour. The mixture is poured dropwise with mixing into a 10% solution of mercuric sulfate in 5% sulfuric acid and stored for 20 hours at room temperature. The precipitate is collected, washed with water

and decomposed with hydrogen sulfide in 150 ml. of aqueous suspension. The solution is filtered, heated to remove hydrogen sulfide, then adjusted to pH 8 with barium hydroxide. The barium sulfate is filtered off, and the solution is concentrated under vacuum to about 2 ml. (Note 3).

(b) *L-Histidine-2-C^{14} Diflavianate.* The crude L-2-mercaptohistidine-2-C^{14} is heated on a water-bath for 1 hour with 8 g. of ferric sulfate in 40 ml. of water. Hot water (200 ml.) and an excess of barium hydroxide are added, and the precipitate is filtered off and washed thoroughly with boiling water. The combined solution is adjusted to pH 4 with sulfuric acid, and the precipitated barium sulfate is filtered off. The filtrate is concentrated to 5 ml., 0.550 g. of added flavianic acid is dissolved with heating, and the product is crystallized by storage at room temperature, and overnight at 0°. The crystals are collected and washed with absolute alcohol and with ether; yield 0.486 g., m.p. 240–245° (Note 4).

(c) *L-Histidine-2-C^{14}.* L-Histidine-2-C^{14} diflavianate (1.244 g.) is dissolved in 12 ml. of 2 *N* sulfuric acid, and the solution is washed with seven portions of warm butyl alcohol. The aqueous solution is decolorized with charcoal and adjusted to pH 7.1 with barium hydroxide. The mixture is filtered, and the filtrate is concentrated under reduced pressure, then transferred to a centrifuge cone and dried in a stream of nitrogen. The product is allowed to crystallize slowly from 0.5 ml. of water (0°); then the mixture is diluted with 2–3 volumes of absolute alcohol, and the product is collected and washed with absolute alcohol and with ether; yield 0.210 g., m.p. 272°(dec.) (Note 5). Another crop is obtained from the mother liquors.

B. Notes

1. The procedure is a modification of the methods of Tesar and Rittenberg,[1] and of Ashley and Harington.[2]

2. The required 1,2-ketoamine for the Wohl-Marckwald synthesis of imidazoles may be prepared conveniently by degradation of L-histidine according to the procedure of Tesar and Rittenberg.[1,2]

3. Schayer has reported a m.p. over 300° for isotopic material prepared by this procedure. Tesar and Rittenberg[1] reported a yield of 32%.

4. Schayer[3] has obtained a yield of 0.370 g. (m.p. 245–247°) from 0.150 g. of the labeled 2-mercaptohistidine by this same procedure.

5. Schayer[3] has realized a yield of 0.0596 g. (m.p. 294°) from 0.370 g. of labeled diflavianate by this procedure, $[\alpha]_D^{20}$–35.5°. Ashley and Harington[2] reported m.p. 295°. Tesar and Rittenberg[1] have reported a 52% yield of histidine (purified as the monohydrochloride instead of the flavianate) based on 6 g. of 2-mercaptohistidine. A degradation is described by Levy.[4]

C. Other Preparations

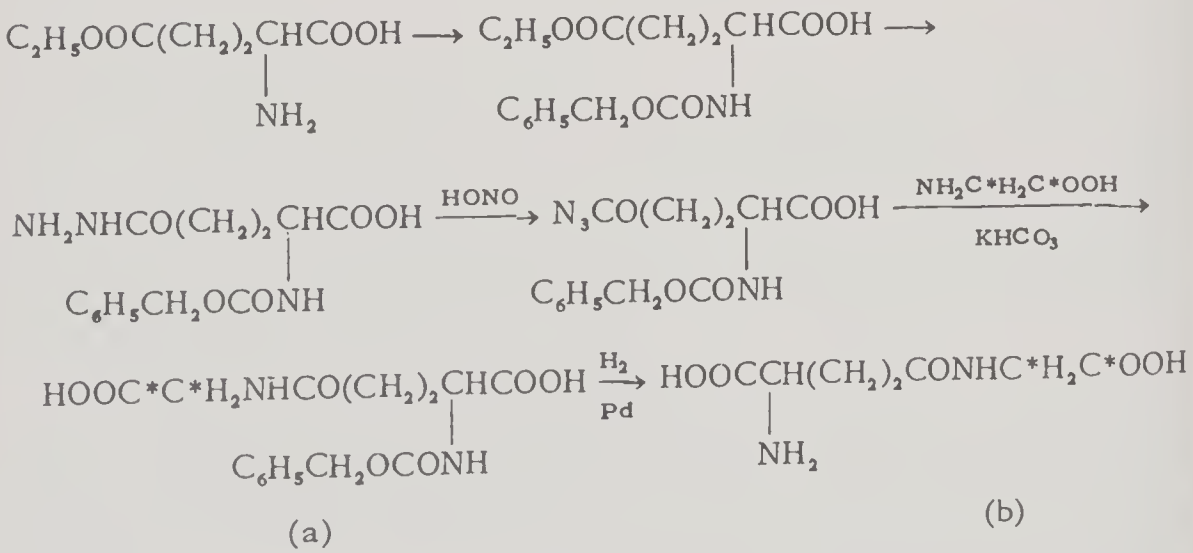
L-Histidine-2-C^{14} has been prepared by Reid[5] in essentially the described manner; over-all yield 7.1%. Purity was checked by paper chromatography. The possibility of partial racemization at the α-position was investigated and found to be less than 1.3%.

The same compound has been prepared by Sprinson[6] by essentially the same method. The product was precipitated as the mercuric chloride complex, then converted to the monohydrochloride hydrate. The yield of crude L-2-mercaptohistidine-2-C^{14} was 1.83 g. from 20 mmoles of potassium thiocyanate-C^{14}.

2-Histidine-2-C^{14} hydrochloride has been prepared by Toporek[7] by a slight modification of this method. The purity was confirmed by nitrogen analysis, optical rotation, homogeneity in paper chromatography (ninhydrin reaction) and radioautography.

[1]C. Tesar and D. Rittenberg, J. Biol. Chem., *170*, 35 (1947).
[2]J. N. Ashley and C. R. Harington, J. Chem. Soc., *1930*, 2586.
[3]R. W. Schayer, J. Am. Chem. Soc., *74*, 2440 (1952).
[4]L. Levy and M. J. Coon, J. Biol. Chem., *208*, 691 (1954).
[5]J. C. Reid, M. O. Landefeld and J. L. Simpson, J. Nat. Cancer Inst., *12*, 929 (1952).
[6]D. B. Sprinson and D. Rittenberg, J. Biol. Chem., *198*, 655 (1952).
[7]M. Toporek, L. L. Miller and W. F. Bale, *ibid.*, *198*, 839 (1952).

N-(CARBOXYMETHYL-C_2^{14}) GLUTAMINE
(*N*-γ-L-Glutamylglycine-C_2^{14})



R. W. Hendler and D. M. Greenberg, Biochem. J., 57, 641 (1954); Nature, *170*, 123 (1952).

A. Procedure

(a) N-(N-*Carbobenzyloxy-γ-L-glutamyl*)*glycine-*C_2^{14} (Note 1). To a vigorously stirred mixture of 0.157 g. of (*N*-carbobenzyloxy-γ-L-glutamyl)-hydrazine (Note 2), 1.94 ml. of water, 0.2 ml. of concentrated hydrochloric acid and 1.35 ml. of ethyl acetate, cooled to 0° (Note 3), is added a solution of 0.0618 g. of sodium nitrite in 0.18 ml. of water during 5 minutes. The mixture is stirred for an additional 10 minutes; then the ethyl acetate layer is separated and washed with two 0.2-ml. portions of cold water. The extract of the azide is added slowly to a cold stirred solution of 0.040 g. of glycine-C_2^{14} and 0.188 g. of potassium bicarbonate in 1 ml. of water (Note 4), the funnel being rinsed with 0.2 ml. of ethyl acetate. The mixture is stirred for 15 minutes in the cold, then stored for 2.5 hours at room temperature and finally for 10–15 minutes at 40° (Note 5). The aqueous layer is acidified with concentrated hydrochloric acid and extracted with four 1-ml. portions of ethyl acetate. The combined extracts are washed with 0.3 ml. of water and evaporated in an air stream. The residue is washed with ether and dried overnight in a vacuum desiccator over phosphorus pentoxide; yield 0.1416 g. Purification is accomplished by recrystallization from 7 drops of boiling water (Note 6) and thorough washing with cold water; yield 0.110 g. (Note 7).

(b) N-(*Carboxymethyl-*C_2^{14})*glutamine* (Note 8). Hydrogen is passed through a stirred mixture of 0.110 g. of N-(*N*-carbobenzyloxy-γ-L-glutamyl)-glycine-C_2^{14}, 0.5 ml. of methanol containing 0.02 ml. of glacial acetic acid and 2 drops of water, and 0.0125 g. of palladous oxide in a microbubbling apparatus. An equal amount of mixed solvent is added at 37 minutes to replace that lost by evaporation. The reaction is complete at about 1 hour when no further carbon dioxide is evolved (Note 9). The catalyst is separated by centrifuging, and the supernatant solution and 0.5 ml. of wash water are filtered and evaporated under reduced pressure (Note 10); yield 0.0524 g. Purification is effected by twice reprecipitating from 15 drops of aqueous solution by the addition of 68 drops of ethanol; yield 0.0262 g., 24% based on glycine (Note 11).

B. Notes

1. The procedure is a modification of the method of LeQuesne.[1] The amount of hydrazide used is increased, raising the yield from 20 to 61% based on glycine.

2. The starting material is prepared[2] by the following steps: DL-glutamic acid,[2] ethyl γ-L-glutamate,[2] ethyl *N*-carbobenzyloxy-γ-L-glutamate,[3,2] and (*N*-carbobenzyloxy-γ-L-glutamyl)hydrazine[1] (m.p. 179–180° from water).

3. The mixture is contained in a 10-ml. separatory funnel fitted with a side arm and immersed in an ice-bath.

4. The reaction is run in a 5-ml. separatory funnel fitted with a side arm and cooled with an ice-bath

5. Ethyl acetate (0.3 ml.) may be added to replace loss by evaporation.

6. A slow rate of cooling is achieved by immersing the container in a very large beaker of hot water.

7. *N*-(*N*-Carbobenzyloxy-γ-L-glutamyl)glycine, recrystallized from hot water, melts at 158–161°.

8. The procedure is that of LeQuesne.[1]

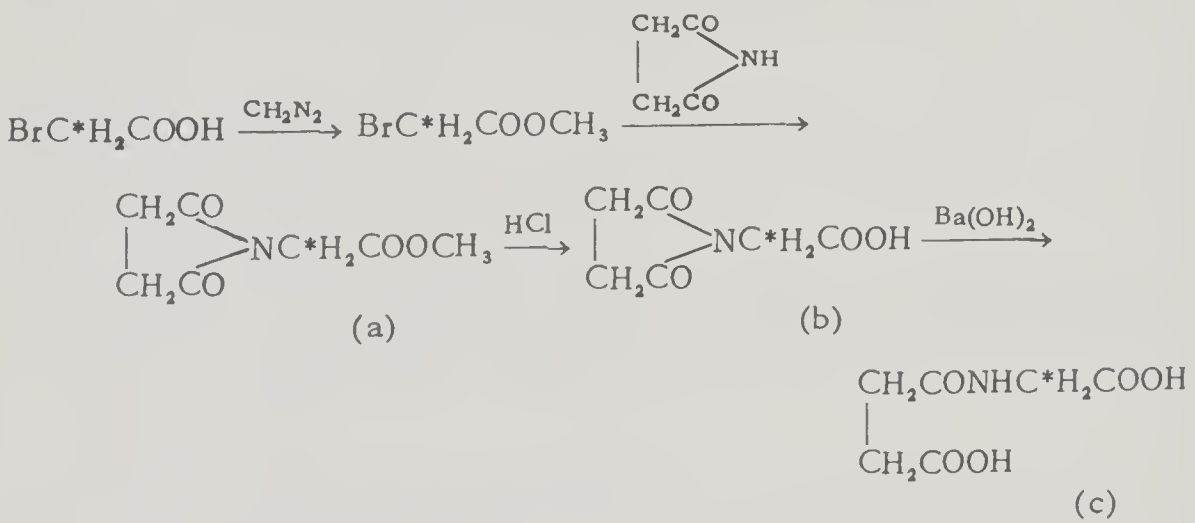
9. From the effluent gas is collected 0.055 g. of barium carbonate equivalent to a maximum yield of 0.057 g. of product.

10. Evaporation is accomplished by immersing the open end of a sealed capillary tube in the solution and placing the container in a vacuum desiccator over phosphorus pentoxide.

11. *N*-γ-L-Glutamylglycine melts at 193–194°, yield 74%.

[1]W. J. LeQuesne and G. T. Young, J. Chem. Soc., *1950*, 1959.
[2]W. E. Hanby, S. G. Waley and J. Watson, J. Chem. Soc., *1950*, 3239.
[3]B. Hegedus, Helv. Chim. Acta, *31*, 737 (1948).

N-CARBOXYMETHYL-C^{14}-SUCCINAMIC ACID



A. Neuberger and J. J. Scott, J. Chem. Soc., *1954*, 1820; Nature, *172*, 1093 (1953).

A. Procedure

(a) *Methyl Succinimidoacetate-2-*C^{14} (Note 1). To a stirred mixture of 21.4 g. (140 mmoles) of methyl bromoacetate-2-C^{14}, 14.5 g. (144 mmoles)

of powdered succinimide[1] and 25 ml. of anhydrous dimethylformamide (Note 2) is added 20 g. (145 mmoles) of anhydrous potassium carbonate in small portions, to minimize opening of the ring. The mixture is stored overnight, then heated at 60° for 1 hour, cooled and diluted with chloroform and water. The chloroform layer is separated and washed with two portions of 5% sodium bicarbonate and water. The washings are extracted with two portions of chloroform, and the combined extract is dried over sodium sulfate and evaporated under reduced pressure (Note 3). The crude product (19.2 g., 80%, m.p. 85–90°) is recrystallized from absolute ether-light petroleum (60–80°); m.p. 96–97° (subl.).

(b) *Succinimidoacetic-2-C^{14} Acid.* A solution of 10.43 g. (61 mmoles) of methyl succinimidoacetate-2-C^{14} in 100 ml. of 7 *N* hydrochloric acid is stored at 37° for 3 days (Note 4), then evaporated under vacuum at 40°. The residue is stored for 2 days under high vacuum over sodium hydroxide, then recrystallized from 500 ml. of benzene; yield 8.61 g. (90%), m.p. 115°.

(c) N-*Carboxymethyl-C^{14}-succinamic Acid, (Succinamidoacetic-2-C^{14} Acid).* To a concentrated aqueous solution of 8.61 g. of succinimidoacetic-2-C^{14} acid is added 1.95 equivalents of barium hydroxide during 2 hours (Note 5). After 1 hour the mixture is heated to 70°. The barium is precipitated with sulfuric acid and separated by centrifuging. The solution is evaporated under vacuum at 50°, and the dried residue is extracted with hot dioxane. The product is crystallized by treating the hot filtered extract with boiling cyclohexane; yield 58% (40% based on bromoacetic-2-C^{14} acid), m.p. 146° after a second recrystallization from dry light petroleum.

B. Notes

1. The method of condensation is an adaptation of that of Sheehan and Bolhofer.[2]

2. Tracds of water lower the yield of Gabriel condensations in this solvent. The material is prepared[3] (80%) from dimethylamine by the *N*-formylation with chloral, which is general for amines.

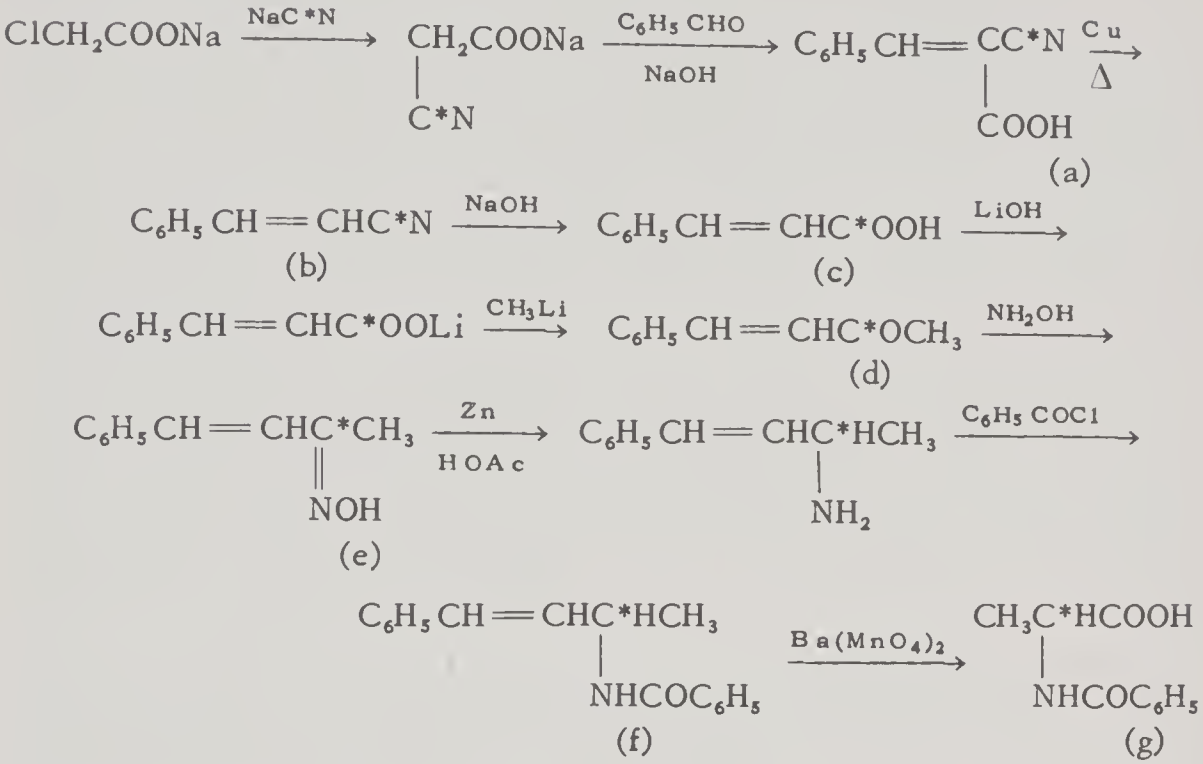
3. Residual dimethylformamide will be removed by evaporation at 90° (14 mm.).

4. Hydrolysis is incomplete after 7 days at 20°.

5. The use of sodium hydroxide appears to form a loosely bound polymer of glycine and succinic acid.

[1] *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 562.
[2] J. C. Sheehan and W. A. Bolhofer, J. Am. Chem. Soc., 72, 2786 (1950).
[3] F. F. Blicke and C. Lu, J. Am. Chem. Soc., 74, 3933 (1952).

N-BENZOYLALANINE-2-C^{13}



J. Baddiley, G. Ehrensvard and H. Nilsson, J. Biol. Chem., *178*, 399 (1949).

A. Procedure

(a) *α-Cyano-C^{13}-cinnamic Acid.* Sodium chloroacetate is condensed with sodium cyanide-C^{13}, and the resulting sodium cyano-C^{13}-acetate is condensed, in turn, with benzaldehyde by the method of Lapworth and Baker;[1] yield 82–85%.

(b) *Cinnamonitrile-C^{13}.* A mixture of 100 g. of crude α-cyano-C^{13}-cinnamic acid and 20 g. of copper powder is heated at 245° until carbon dioxide evolution ceases (10 minutes); then the residue is rapidly vacuum-distilled. The yield of crude product, containing a little water, is 85%.

(c) *Cinnamic-C^{13} Acid, (3-Phenylacrylic-1-C^{13} Acid).* A mixture of 14 g. of crude cinnamonitrile-C^{13}, 115 ml. of 33% sodium hydroxide and a little alcohol is refluxed until the evolution of ammonia ceases. The mixture is diluted with water, the pH is adjusted to 1 with sulfuric acid, and the product is filtered off, washed with water and dried; yield 82%, m.p. 130° (Note 1).

(d) *4-Phenyl-3-buten-2-one-2-C^{13}, (Methyl Styryl Ketone-C^{13})* (Note 2). An aqueous solution of cinnamic-C^{13} acid containing one equivalent of lithium hydroxide is evaporated to dryness, and the dry salt is washed with absolute ether and powdered. To a stirred suspension of the lithium salt in absolute ether is added, in a slow stream of dry nitrogen, a filtered solution of 1.20 molar amounts of methyllithium[2] in absolute ether. The mixture is refluxed for 6 hours and poured onto ice. The ether layer is

separated, washed with water and dried over sodium sulfate, and the solvent is distilled; yield 90–95%, m.p. 40–42°.

(e) *4-Phenyl-3-buten-2-one-2-C*^{13} *Oxime.* To an alcoholic solution of the ketone is added a concentrated aqueous solution of 1.2 molar amounts of hydroxylamine hydrochloride and sodium acetate. The mixture is refluxed for 4 hours, stored overnight at room temperature, then concentrated under vacuum to small volume. The residue is diluted with water, and the product is filtered off and air-dried; yield 97%, m.p. 89°.

(f) N-(*α-Methylcinnamyl-α-C*^{13}*)benzamide* (Note 3). A mixture of 40 g. of the oxime in 300 ml. of absolute ethanol and 300 ml. of glacial acetic acid is reduced at 0° with 100 g. of zinc dust, added in small portions. The mixture is finally warmed on a water-bath for 30 minutes and filtered, and the alcohol is distilled. The mixture is basified with sodium hydroxide, and the 3-amino-1-phenyl-1-butene-3-C^{13} is steam-distilled and treated with benzoyl chloride and sodium hydroxide by the Schotten-Baumann method. The yield of product is 40–45%, m.p. 137° after recrystallization from alcohol.

(g) N-*Benzoylalanine-2-C*^{13}. To a stirred mixture of 2.5 g. of the amide and 20 ml. of pyridine, maintained at 40° throughout the reaction, are added in alternate small portions 5 g. of barium permanganate in 40 ml. of water and 5 g. of barium hydroxide in 50 ml. of warm water. The addition is stopped when a faint-pink color persists. The manganese dioxide is removed by filtration and thoroughly washed with hot water. The combined filtrate and washings are warmed with a few drops of methanol to destroy excess permanganate, then treated with carbon dioxide until the solution is only weakly alkaline. The mixture is filtered, and the filtrate is evaporated to dryness under vacuum. The residue is dissolved in 50% alcohol, and the barium is precipitated by the addition of 4 *N* sulfuric acid (Note 4). The solution is filtered and concentrated to a small volume. The benzoic acid is removed by ether extraction, and the product is crystallized from the aqueous solution; yield 65%, m.p. 163° (Note 5).

B. Notes

1. Methyl cinnamate-C^{14} and -α-C^{14} have been prepared by Ropp[3] from sodium acetate-1-C^{14} and -2-C^{14}, respectively, by use of the Perkin reaction[4] employing benzaldehyde and acetic anhydride. No isotopic effect was found in the bromination of the esters.

2. The procedure is adapted from the method of Gilman and Van Ess.[5] Low yields of 4-phenyl-3-buten-2-one are obtained by conversion of cinnamonitrile by the Grignard method,[6] or by treating cinnamoyl chloride with methylcadmium,[7] or by treating cinnamic acid with methyllithium.[2]

3. The free base, α-methylcinnamylamine-α-C^{13} is prepared by the method of Harries and de Osa.[8]

4. Rhodizonic acid may be used as indicator.

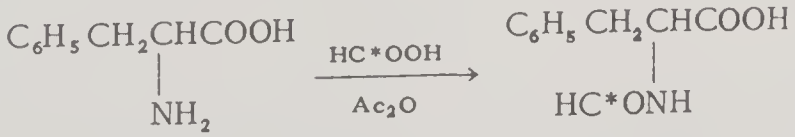
5. The yield on a 120-g. scale with calcium permanganate was 55%. Pure L- and D-alanine-2-C^{13} have been obtained by resolution through the strychnine and brucine salts followed by hydrolysis, according to the method of Pacsu and Mullen.[9] The respective yields, however, are less than the 68% and 64% claimed.

C. Other Preparations

DL-Alanine-2-C^{14} has been prepared from pyruvic-2-C^{14} acid by Anker.[10] The phenylhydrazone, prepared in 95% yield by treating the acid with an excess of phenylhydrazine hydrochloride, was quantitatively hydrogenated over Adams catalyst in water suspension at room temperature and atmospheric pressure. Alkali was added, and the aniline formed was removed with ether. The aqueous solution was treated with benzoyl chloride; then the optical antipodes were separated by the brucine-strychnine salts method of Fischer.[11] D-alanine-2-C^{14}, $[\alpha]_D -12.9°$; L-alanine-2-C^{14}, $[\alpha]_D +12.5°$.

[1]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 181.
[2]D. A. van Dorp and J. F. Arens, Rec. trav. chim., *65*, 338 (1946).
[3]G. A. Ropp, V. F. Raaen and A. J. Weinberger, J. Am. Chem. Soc., *75*, 3694 (1953).
[4]*Organic Reactions*, Vol. I, Wiley, 1942, Chapter 8.
[5]H. Gilman and P. R. Van Ess, J. Am. Chem. Soc., *55*, 1258 (1933).
[6]E. P. Kohler, Am. Chem. J., *35*, 403 (1906).
[7]H. Gilman and J. F. Nelson, J. Am. Chem. Soc., *55*, 518 (1936).
[8]C. Harries and A. S. de Osa, Ber., *36*, 2997 (1903).
[9]E. Pacsu and J. W. Mullen, 2nd., J. Biol. Chem., *136*, 335 (1940).
[10]H. S. Anker, J. Biol. Chem., *187*, *167* (1950).
[11]E. Fischer, Ber., *32*, 2451 (1899).

N-FORMYL-C^{14}-L-3-PHENYLALANINE



V. du Vigneaud, W. G. L. Verly, J. E. Wilson, J. R. Rachele, C. Ressler and J. M. Kinney, J. Am. Chem. Soc., *73*, 2782 (1951).

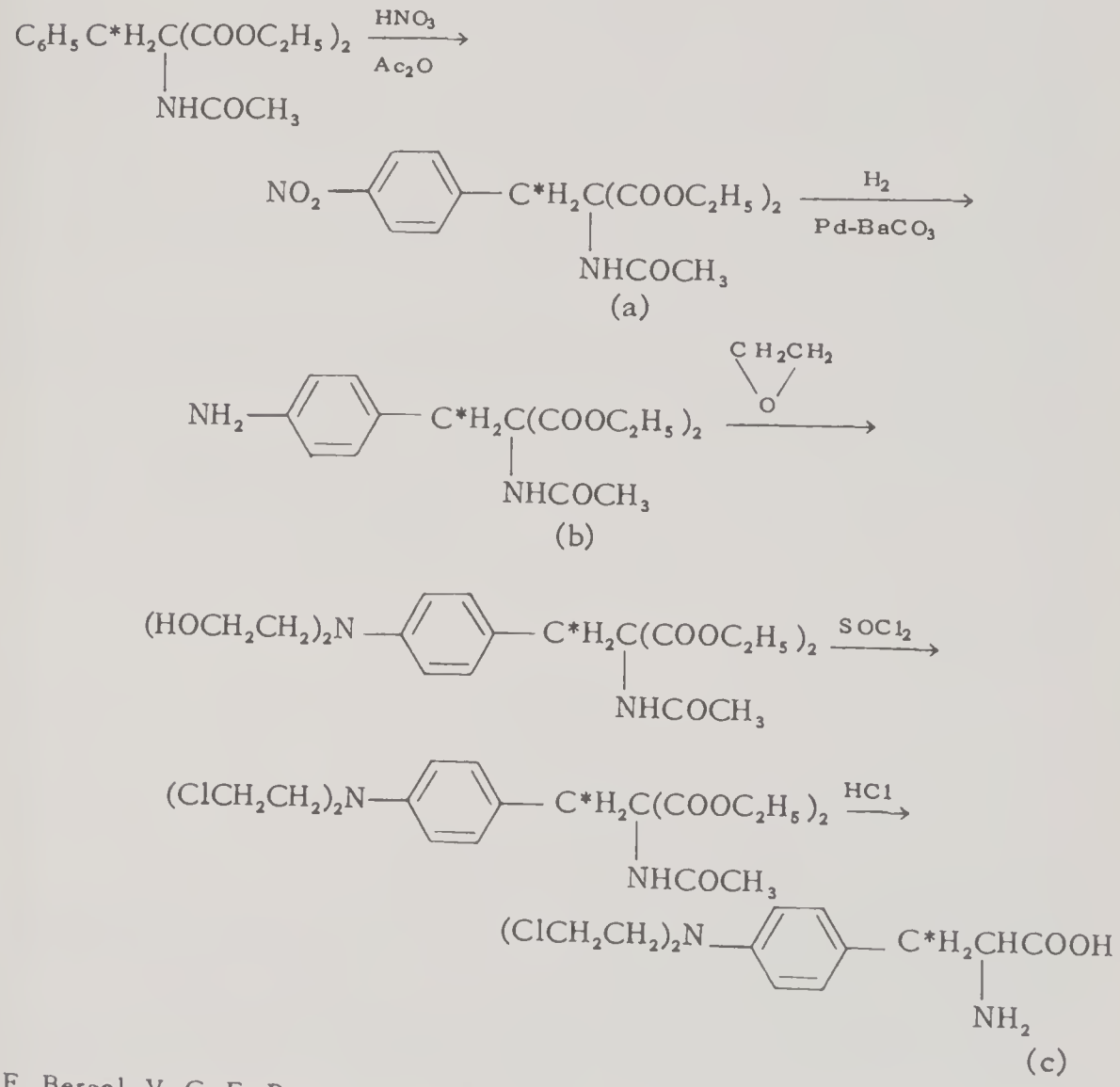
Procedure

L-3-Phenylalanine is formylated by a modification of the method of du Vigneaud and Meyer.[1] A mixture of 0.31 mmole of sodium formate-C^{14}, 3.16 mmoles of formic acid and 2 drops of 85% phosphoric acid is set aside for 4 hours at room temperature. The mixture is distilled, in a closed evacuated system, into a test-tube cooled with Dry Ice. Acetic

anhydride (0.6 ml.) is added to the distillate, which is set aside for 2.5 hours and then mixed with 3.4 mmoles of L-phenylalanine. After another 2.5 hours, all valatile liquid is removed by vacuum distillation, and the residue is washed with two portions of *N*-hydrochloric acid and once with water. After two recrystallizations from water, the yield of colorless needles is 55%, m.p. 171–172°.

[1]V. du Vigneaud and C. E. Meyer, J. Biol. Chem., *98*, 295 (1932).

3-[*p*-BIS(2-CHLOROETHYL)AMINOPHENYL]ALANINE-3-C^{14}



F. Bergel, V. C. E. Burnop and J. A. Stock, J. Chem. Soc., *1955*, 1223.

A. Procedure (Note 1)

(a) *Ethyl Acetamido-(p-nitrobenzyl-α-C^{14})malonate.* To a stirred solution of 0.611 g. of ethyl acetamidobenzyl-α-C^{14} malonate in 1.6 ml. of acetic anhydride at 0° is added 0.40 ml. of fuming nitric acid dropwise during 5 minutes. The mixture is stored at room temperature for 2 hours,

then mixed with 5 g. of ice, and the product is collected and recrystallized twice from ethyl methyl ketone; yield 0.231 g., 45% (Note 2). An additional crop (0.227 g.) is obtained by recrystallizing 0.231 g. of carrier from the mother liquor.

(b) *Ethyl Acetamido-*(p-*aminobenzyl-*α-C^{14}*)malonate.* A mixture of 0.458 g. of ethyl acetamido-(*p*-nitrobenzyl-α-C^{14})malonate and 10 ml. of ethyl acetate is hydrogenated in the presence of palladium-barium carbonate. The product is recrystallized from ethyl acetate-light petroleum; yield 0.368 g., 87% (Note 3).

(c) 3-[p-*Bis(2-chloroethyl)aminophenyl]alanine-*3-C^{14} (Note 3). To a solution of 0.368 g. of ethyl acetamido-(*p*-aminobenzyl-α-C^{14})malonate, in 1 ml. of glacial acetic acid is added 3 ml. of 20% aqueous ethylene oxide. The mixture is stored for 24 hours, then diluted with 10 ml. of water and neutralized with solid sodium bicarbonate (2 g.). The precipitated ethyl acetamido-[*p*-bis(2-hydroxyethyl)aminobenzyl-α-C^{14}]-malonate is extracted with three 5-ml. portions of chloroform. The solution is filtered through anhydrous sodium sulfate and dried azeotropically by distillation to one-half volume (Note 4).

The solution is refluxed for 10 minutes with 0.5 ml. of thionyl chloride, then diluted with 5 ml. of concentrated hydrochloric acid, and the ethyl acetamido-[*p*-bis(2-chloroethyl)aminobenzyl-α-C^{14}]malonate is hydrolyzed by refluxing the mixture for 3 hours (Note 5). The mixture is evaporated under vacuum, the residue is dissolved in 5 ml. of water (Norit), and the product is precipitated by the addition of sodium acetate; yield 0.315 g. (89%). Purification is accomplished, just before use, by recrystallization from methanol; radiochemical yield 22% based on barium carbonate, m.p. 177–178° (dec.) (Note 6).

B. Notes

1. The procedure is an adaptation of that of Bergel and Stock.[1]

2. The product in trial preparations melted at 192–194°. Although the yield is low, the alternative routes through the *p*-nitro-, *p*-acetamido-, and *p*-phthalimidobenzonitrile were less satisfactory based on C^{14}-cyanide.

A method of preparing the pure D- and L-forms of the final product (c) from DL-3-(*p*-nitrophenyl)alanine is described by Bergel and Stock.[1]

3. Ethyl acetamido-(*p*-aminobenzyl)malonate melts[2] at 132°.

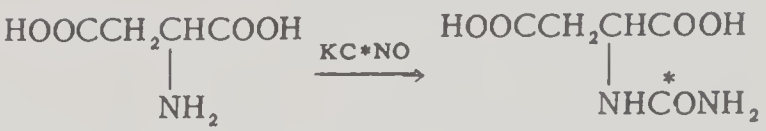
4. Ethyl acetamido-[*p*-bis(2-hydroxyethyl)aminobenzyl]malonate melts[1] at 100°.

5. An air condenser is used to allow the escape of chloroform.

6. A pure sample melts at 181–182° (dec.). The compound is readily dehalogenated under alkaline conditions.

[1]F. Bergel and J. A. Stock, J. Chem. Soc., *1954*, 2409.
[2]D. F. Elliott and C. Harington, J. Chem. Soc., *1949*, 1374.

N-CARBAMOYL-C^{14}-ASPARTIC ACID
(Ureido-C^{14}-Succinic Acid)



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A. Procedure (Note 1)

A mixture of 2.5 g. (31 mmoles) of potassium cyanate-C^{14} and 4.1 g. (31 mmoles) of aspartic acid is dissolved in 40 ml. of *N* potassium hydroxide and transferred to a flask by washing with 2 ml. of the solvent. The solution is stored at room temperature for 48 hours, then cooled in an ice-bath and acidified to pH 2 by the dropwise addition of concentrated hydrochloric acid. The mixture is stored at room temperature for 24 hours and then in a refrigerator to crystallize the product; yield 2.9 g. (55% based on potassium cyanate), m.p. 180–181°. Chromatography in a system of ethyl acetate, methanol, 98% formic acid and water (6:6:3:5 v/v) indicates the presence of only one radioactive compound. The absence of unreacted amino acid starting material is indicated by a negative ninhydrin color reaction. The molar specific activity agrees with that of the starting material.

B. Notes

1. The procedure is a modification of that of Nyc and Mitchell.[1] Increasing the reaction time and acidifying at low temperature give more reproducible yields. Use of the original procedure has been reported by Weed.[2]

C. Other Preparations

A similar preparation of *N*-carbamoyl-C^{14}-aspartic acid has been reported by Weed;[2] yield 68% at the 100-mmole level, m.p. 178–180° after recrystallization from water.

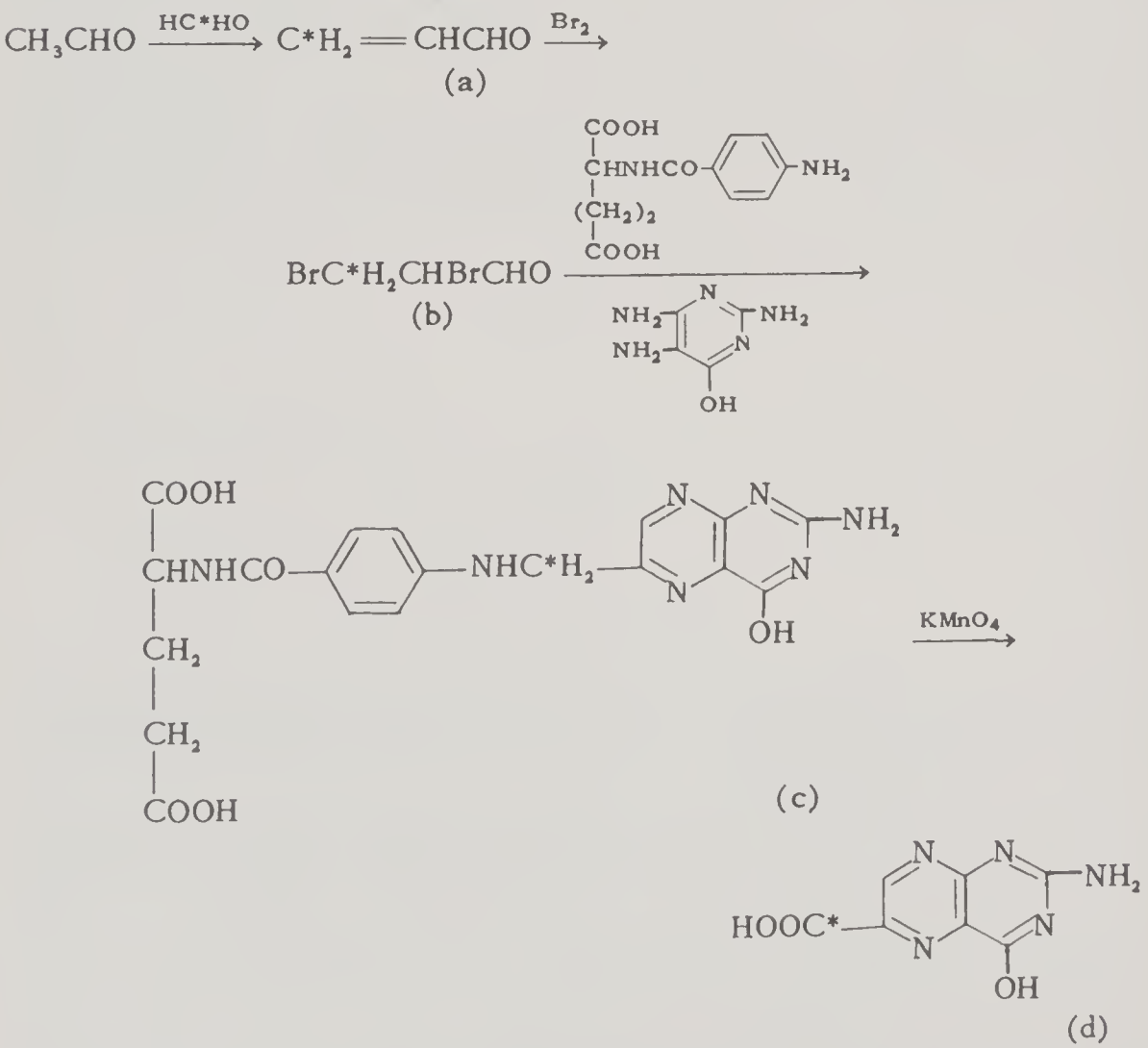
The preparation of the D- and L-forms, as well as the DL-*N*-carbamoyl-C^{14}-aspartic acid, from the corresponding isomers of aspartic acid has been reported by Lieberman.[3] Purification was accomplished by ion exchange chromatography (Dowex-1 and 0.055 *M* sodium formate adjusted to pH 3.2 with formic acid), since crystallization of the optically active product was unsuccessful.

N-Carbamoylaspartic-C^{14} acid has been prepared[4] by condensing aspartic-4-C^{14} acid with potassium cyanate at pH 5.5 for 18 hours.

N-Carbamoyl-C^{14}-L-glutamic acid has been prepared[3] by a similar procedure,[5] m.p. 158–160° from water.

[1]J. F. Nyc and H. K. Mitchell, J. Am. Chem. Soc., 69, 1382 (1947).
[2]L. L. Weed and D. W. Wilson, J. Biol. Chem., *189*, 435 (1951); L. D. Wright, C. S. Miller, H. R. Skeggs, J. H. Huff, L. L. Weed and D. W. Wilson, J. Am. Chem. Soc., *73*, 1898 (1951).
[3]I. Lieberman and A. Kornberg, J. Biol. Chem., *207*, 911 (1954); *212*, 909 (1955).
[4]D. W. Wilson, Nucleonics, 7 (4), 51 (1950).
[5]P. P. Cohen and S. Grisolia, J. Biol. Chem., *182*, 747 (1950).

N-{*p*-[(2-AMINO-4-HYDROXY-6-PTERIDYLMETHYL-C^{14})AMINO]-BENZOYL}-L-GLUTAMIC ACID (C_1^{14}-Folic Acid)



F. Weygand and G. Schaefer, Naturwiss., 38, 432 (1951); Ber., 85, 307 (1952).

A. Procedure

(a) *Acrolein-3-C*^{14} (Note 1). The apparatus (Figure II, 10) consists of a 30-cm. pressure-equalizing, burette (Note 2) attached to a vertical catalyst column (50 × 1.7 cm. i.d.), which contains 1 to 2-mm. granules of 10% sodium silicate on silica gel overlying a 10-cm. layer of glass-wool. A 280° range thermometer is embedded to a depth of 30 cm. in the catalyst mass, which is topped off with a short plug of glass-wool and heated by a 35-cm. electric furnace. The outlet is connected, in turn, to a short coil condenser which is cooled for three-fourths of its length by standing water (Note 3), a Dry Ice-cooled trap (50 ml.) containing 20–30 mg. of hydroquinone, and a trap cooled with liquid nitrogen.

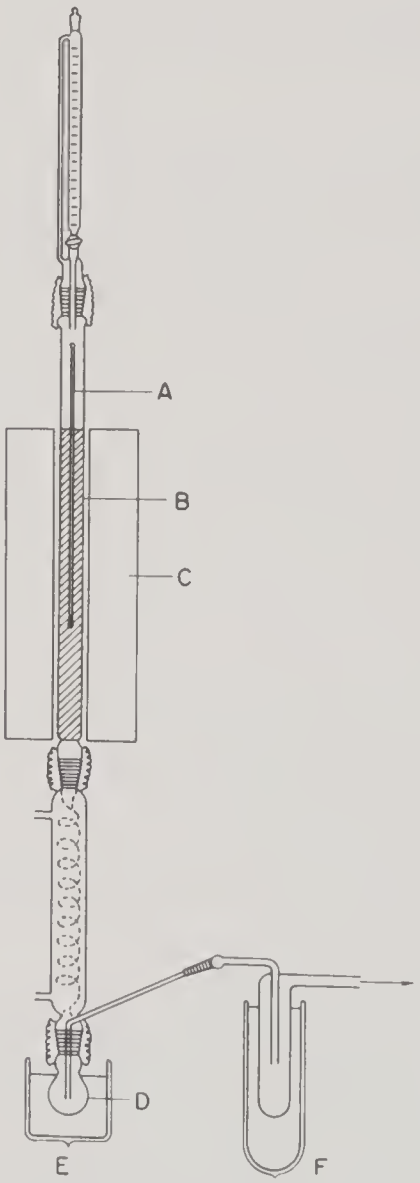


Fig. II, 10. Apparatus for preparation of acrolein (F. Weygand and G. Schaefer). A, thermometer; B, catalyst; C, electric oven; D, receiver; E, Dry Ice-acetone bath; F, liquid nitrogen.

A mixture of 4.75 g. of formaldehyde-C^{14} (26% aqueous solution) and 7.1 g. of acetaldehyde (equimolecular amounts) is dropped at a constant rate of 19.2 ml. per hour onto the catalyst heated to 295° (Note 4). At the end of the reaction, 6 ml. of water is added similarly; yield 3.5 g., 39–39.5% (Note 5).

(b) 2,3-*Dibromopropionaldehyde-3-*C^{14}. The crude acrolein-3-C^{14} mixture is diluted with 4 ml. of ether, cooled in a freezing mixture and treated dropwise with bromine (9.9 ml.) so that the mixture is decolorized in 30–45 seconds. The addition is ended when a pale yellow color persists.

(c) N-{p-[(2-*Amino-4-hydroxy-6-pteridylmethyl-*C^{14}*)amino]benzoyl*}-*L-glutamic Acid* (Note 6). To a vigorously stirred solution of 30 g. of 2,5,6-triamino-4-pyrimidinol sulfate, 30 g. of *N-p*-aminobenzoyl-L-glutamic acid and 20 g. of crystalline sodium acetate in 1.5 l. of water, adjusted to and maintained at pH 4.0 with *N* sodium hydroxide (Note 7), is added dropwise over a period of 1.5 hours a solution of the crude 2,3-dibromopropionaldehyde-3-C^{14}, 5.5 g. of quinone, and 1.5 l. of ethanol. The mixture is stirred for 1 hour and stored overnight. The brick-red precipitate is filtered off and washed with water and alcohol, then made into a paste with 50 ml. of 2 *N* sodium hydroxide in a mortar and poured into 1.2 l. of water. The addition of 40 ml. of 2 *N* sodium hydroxide completely dissolves the material, and the addition of 12 g. of crystalline barium chloride in a little water then forms a brown precipitate, which is filtered off with the aid of diatomaceous earth. The filtrate is adjusted to pH 7 with 2 *N* hydrochloric acid, heated to boiling, treated with 5 g. of diatomaceous earth and filtered hot. The residue is extracted with 4 l. of boiling water, followed by 3 l. of water. The three filtrates are adjusted to pH 3.5 with acetic acid, cooled and stored overnight. The combined pale-yellow precipitate is dissolved in 4 l. of boiling water by the addition of *N* sodium hydroxide to pH 7; then the hot solution is treated with 10 g. of diatomaceous earth and filtered. The filtrate is adjusted to pH 3.5 with hydrochloric acid and slowly cooled overnight to crystallize; yield 1.9 g., 11.1% based on acrolein (Note 8). Degradation studies show that there is no isotopic rearrangement (see below).

(d) *2-Amino-4-hydroxy-6-pteridinecarboxylic-*C^{14} *Acid.* A stirred solution of 0.5 g. of the C_1^{14}-folic acid in 50 ml. of water and 10 ml. of 2 *N* sodium hydroxide is treated dropwise with 50 ml. of 2% potassium permanganate solution. The mixture is decolorized with 2 ml. of ethanol and filtered hot. The filtrate is acidified to pH 1 with concentrated hydrochloric acid, then adjusted to pH 3 with sodium acetate and cooled to crystallize. The product is collected by centrifuging, washed with water and alcohol, and dried over sodium hydroxide-sulfuric acid; yield 0.226 g. (Note 9). The molar specific activity is the same as that of the folic acid. Degradation studies show that isotope is present only in the carboxyl group (Note 10).

B. Notes

1. The procedure is an adaptation of the method of Schulze and Wagner.[1]

2. The stopcock handle is lengthened for easier fine-adjustment, and the burette is equipped with a capillary drip tip.

3. If flowing water is used, the coil becomes plugged with a polymer of unreacted formaldehyde.

4. Experiment shows that the reaction is exothermic and that the optimum operating temperature is 300–310°.

5. The yield is based upon titration of the crude product from thirteen similar trial runs. The by-products in the commercial preparation are small amounts of methanol, formic acid, methyl formate, crotonaldehyde, carbon monoxide, carbon dioxide and traces of hydrocarbon, as well as appreciable amounts of unreacted starting materials. The crude mixture is used without delay in the following steps with no adverse effects on yields.

6. The procedure is a modification of the methods of Waller[2] and of Doub.[3] Optimum yield is attained with a 100% molar excess of both the pyrimidine and the glutamic acid derivative. According to German nomenclature, the compound is folic-11-C^{14} acid.

7. Considerable study[2] has been devoted to achieving maximum yield by studying the effect of pH, solvents and mode of addition of reactants.

8. The needles formed are the dihydrate. Purity is determined by bioassay. The first crude samples contain about 20% folic acid,[2] but the recovery of product during purification is high. A review of the chemistry of folic acid is presented by Hutchings and Mowat.[4] The purification of folic acid by ion-exchange chromatography has been described by Heinrich.[5]

9. The product is a hemi-hydrate displaying a blue fluorescence.[6] It may also be obtained by aerobic alkaline hydrolysis.[7]

10. The acid is decarboxylated[6] (61% yield) by heating at 260–290° for 4 hours in a stream of nitrogen. The evolved carbon dioxide has a specific activity that is 98.2% of that of the folic acid. Paper chromatographic separation of the residue shows the presence of nonisotopic 2-amino-4-pteridinol.

[1]H. Schulze and H. Wagner, Angew. Chem., *62*, 105 (1950).

[2]C. W. Waller, B. L. Hutchings, J. H. Mowat, E. L. R. Stokstad, J. H. Boothe, R. B. Angier, J. Semb, Y. SubbaRow, D. B. Cousulic, M. J. Fahrenbach, N. E. Hultquist, E. Kuh, E. H. Northey, D. R. Seeger, J. P. Sickels and J. M. Smith, Jr., J. Am. Chem. Soc., *70*, 19 (1948); Science, *103*, 667 (1946).

[3]L. Doub and L. L. Bambas, U. S. 2,476,360; Chem. Abstracts, *44*, 173 (1950).

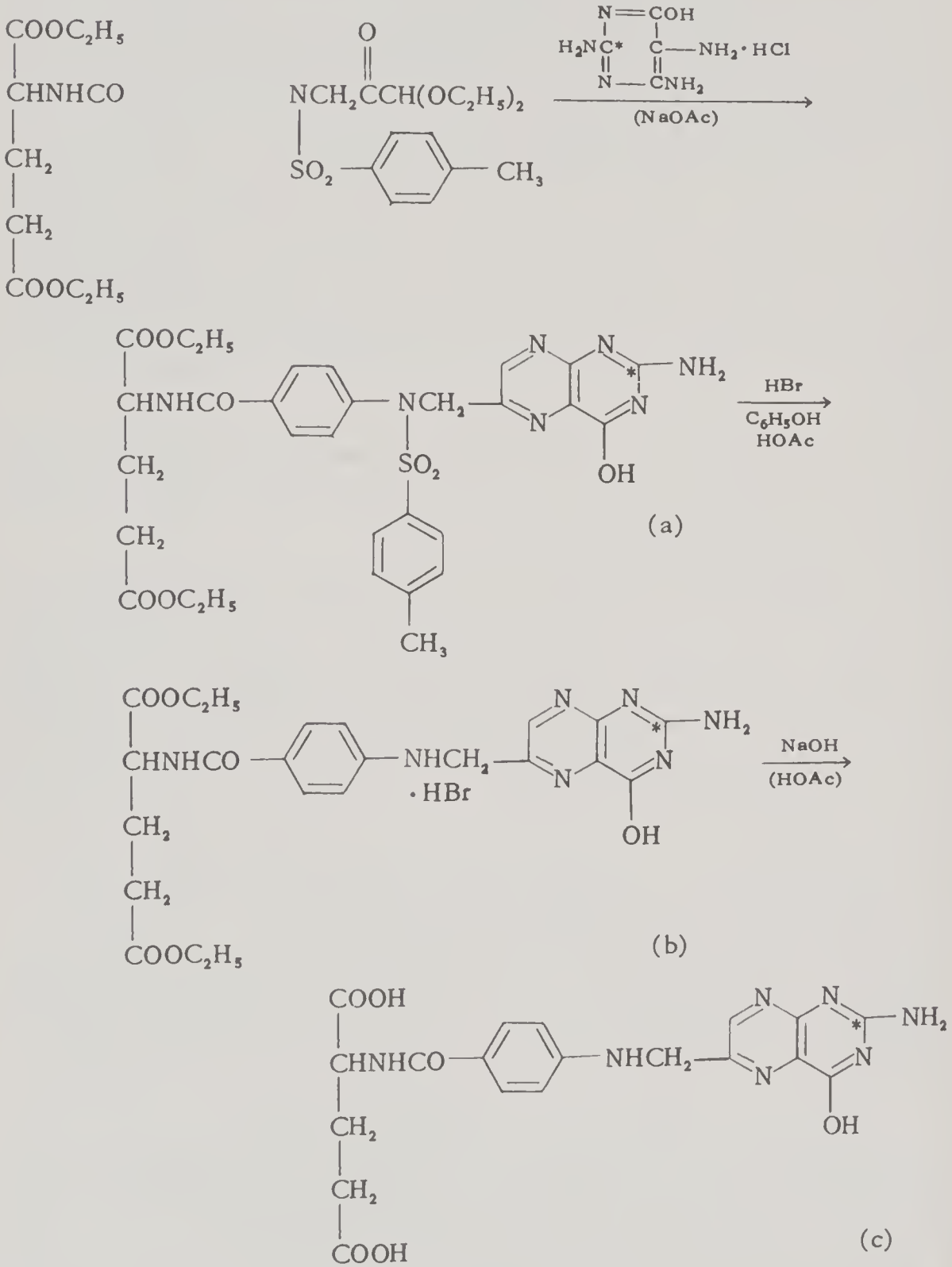
[4]*Vitamins and Hormones*, *6*, 1 (1948); M. Gates, Chem. Rev., *41*, 63 (1947).

[5]M. R. Heinrich, V. C. Dewey and G. W. Kidder, J. Am. Chem. Soc., *75*, 5425 (1953).

[6]F. Weygand, A. Wacker and V. Schmied-Kowarzik, Experientia, *6*, 184 (1950).

[7]E. L. R. Stokstad, B. L. Hutchings, J. H. Mowat, J. H. Boothe, C. W. Waller, R. B. Angier, J. Semb and Y. SubbaRow, J. Am. Chem. Soc., *70*, 5 (1948).

N-{*p*-[(2-AMINO-4-HYDROXY-6-PTERIDYLMETHYL-2-C^{14})AMINO]-BENZOYL}-L-GLUTAMIC ACID
(C_1^{14}-Folic Acid)



F. Weygand, H. Grisebach and H. Simon, Ber., 86, 1389 (1953).

A. Procedure (Note 1)

(a) *Ethyl* N-{p-[N-(2-*Amino-4-hydroxy-6-pteridylmethyl-2-*C^{14})-p-*toluenesulfonamido]benzoyl*}-*L-glutamate.* To the 2,5,6-triamino-4-pyrimidinol-2-

C^{14} hydrochloride, prepared from 1.1 g. of the sulfate (Note 2), is added 0.77 g. of anhydrous sodium acetate and a solution of 2.94 g. of ethyl *N*-[*N*-*p*-toluenesulfonyl-*N*-(2-oxo-3,3-diethoxypropyl)-*p*-aminobenzoyl]-L-glutamate in 27 ml. of glacial acetic acid. The mixture is stirred in the dark in a nitrogen atmosphere at room temperature for 45 minutes, then refluxed for 2 hours, and the solvent finally is removed by vacuum distillation.

(b) *Ethyl* N-{p-[(2-*Amino-4-hydroxy-6-pteridylmethyl*) *amino-2-*C^{14}]*benzoyl*}-*L-glutamate Hydrobromide.* The tosylate is shaken vigorously with 17 ml. of glacial acetic acid saturated with hydrogen bromide and containing 0.85 g. of phenol (Note 3). After standing for 2 hours at 20°, the mixture is diluted with 100 ml. of absolute ether, and the product is collected on a sintered-glass funnel and washed with several portions of ether.

(c) N-{p-[(2-*Amino-4-hydroxy-6-pteridylmethyl-2-*C^{14})*amino*]*benzoyl*}-*L-glutamic Acid,* (*N-Pteroyl-2-*C^{14}*-L-glutamic Acid*). The hydrobromide is dissolved at once in a little methanol and hydrolyzed by adding 20 ml. of 10% sodium hydroxide solution and storing overnight. The solution is diluted with 100 ml. of water and neutralized with concentrated hydrochloric acid. The crude product is precipitated by the addition of 20 ml. of glacial acetic acid, centrifuged and washed with two portions of water, followed by acetone. The product is dissolved in 11. of boiling water, and the filtered solution (Note 4) is neutralized with 2 *N* sodium hydroxide, treated with 5 g. of crystalline sodium acetate and 5 g. of Fuller's earth (Note 5), and filtered. To the filtrate is added an amount of hydrochloric acid equivalent to the sodium acetate, followed by a few ml. of acetic acid. After storage at 0° for a few hours, the product is collected, and the purification procedure is repeated. The crystalline product is dissolved in 50 ml. of boiling water containing 10 g. of sodium acetate and is treated with 5 g. of Fuller's earth. The hot solution is filtered, and the precipitate is washed with a solution of 2 g. of sodium acetate in 200 ml. of hot water. To the combined filtrate is added an amount of hydrochloric acid equivalent to the sodium acetate, followed by a little acetic acid, with cooling, to crystallize the product; yield about 0.400 g., 18% (Note 6).

B. Notes

1. The procedure is adapted from the method of Weisblat and Magerlein,[1] who also describe the preparation of the nonisotopic starting material. Weisblat and coworkers have studied many factors of this reaction.

2. The salt is treated in 50 ml. of hot aqueous solution with 1.08 g. of barium chloride dihydrate in 10 ml. of water. The filtered solution is evaporated to dryness.

3. Phenol functions as a bromine acceptor to prevent simultaneous bromination of the cleavage product.[2]

4. An insoluble brownish impurity is removed.

5. Frankonite KL.

6. The yield was higher with trial runs on a larger scale. For a review of the chemistry of folic acid, see *N*-{*p*-[(2-amino-4-hydroxy-6-pteridylmethyl-C^{14})amino]benzoyl}-L-glutamic acid, ref. 4.

C. Other Preparations

N-Pteroyl-2-C^{14}-L-glutamic acid has been prepared in 3.3% yield by Weygand.[3] *N*-*p*-Aminobenzoyl-L-glutamic acid, 2,5,6-triamino-4-pyrimidinol-2-C^{14} and 1,1,3-tribromo-2-propanone were condensed[4] together in dilute alcohol solution. Purification was achieved by chromatography on a filter plate of linters.

N-Pteroyl-2-C^{14}-L-glutamic acid has been prepared in like manner (1% over-all yield, greater than 90% purity) by Fong.[5] The compound obtained by condensing *N*-*p*-aminobenzoyl-L-glutamic acid with 1,1,3-tribromo-2-propanone was added to a solution of 2,5,6-triamino-4-pyrimidinol-2-C^{14}. Purification of the product was accomplished through precipitation of the zinc and magnesium salts.

N-Pteroyl-2-C^{14}-L-glutamic acid has been prepared by Skipper,[6] with some difficulty in effecting satisfactory purification. An aqueous solution of 2,5,6-triamino-4-pyrimidinol-2-C^{14} hydrochloride and *N*-*p*-aminobenzoyl-L-glutamic acid was treated with an alcohol solution of 2,2,3-tribromopropionaldehyde; see *N*-{*p*-[(2-amino-4-hydroxy-6-pteridylmethyl-C^{14})amino]benzoyl}-L-glutamic acid.

[1]D. I. Weisblat and B. J. Magerlein, U. S. 2,631,149; Chem. Abstracts, *48*, 1446 (1954).

[2]D. I. Weisblat, B. J. Magerlein and D. R. Meyers, U. S. 2,562,222; Chem. Abstracts, *46*, 3093 (1952).

[3]F. Weygand, H. J. Mann and H. Simon, Ber., *85*, 463 (1952).

[4]M. E. Hultquist and P. F. Dreisbach, U. S. 2,443,165; Chem. Abstracts *40*, 7192 (1948); F. Weygand and V. Schmied-Kowarzik, Ber., *82*, 333 (1949).

[5]C. T. Fong, C. M. Judson, N. H. Marsh, D. J. Salley, Nucleonics, 7 (4), 49 (1950).

[6]H. E. Skipper, Nucleonics, 7 (4), 49 (1950).

5-AMINOVALERIC-5-C^{14} ACID

$$Br(CH_2)_3COOC_2H_5 \xrightarrow{KC^*N} NC^*(CH_2)_3COOC_2H_5 \xrightarrow[Ac_2O]{PtO_2}$$

$$CH_3CONHC^*H_2(CH_2)_3COOC_2H_5 \xrightarrow{HCl} HCl \cdot H_2NC^*H_2(CH_2)_3COOH \xrightarrow{Resin}$$

$$NH_2C^*H_2(CH_2)_3COOH$$

M. Rothstein, J. Am. Chem. Soc., 76, 3038 (1954); Atomic Energy Commission Report, UR-323.

A. Procedure

(a) *Ethyl 4-Cyano-C^{14}-butyrate.* A mixture of 0.803 g. (12.4 mmoles) of potassium cyanide-C^{14}, 0.008 g. of potassium hydroxide, 2.5 g. (12.8 mmoles) of ethyl 4-bromobutyrate (Note 1), 22 ml. of 95% ethanol and 11 ml. of water is refluxed for 14 hours under a slow stream of nitrogen, which is exhausted through a trap containing 10 ml. of 10% sodium hydroxide (Note 2). The reaction mixture is distilled to dryness under vacuum, the distilling head is washed with absolute alcohol, and the mixture is again taken to dryness. The residual salt is separated and washed with four portions (total 40 ml.) of absolute ether; then the combined filtrate is evaporated in a gentle air stream. The oily residue is decolorized in 20 ml. of ethanol solution by boiling for 10 minutes with Norit, filtering and evaporating the solvent (Note 3).

(b) *5-Aminovaleric-5-C^{14} Acid.* A mixture of the ethyl 4-cyano-C^{14}-butyrate, 28 ml. of acetic anhydride and 0.25 g. of platinum oxide is hydrogenated at 40° for 18 hours at 50 p.s.i. (Note 4). The filtered solution is evaporated to dryness under vacuum, and the residue is hydrolyzed for 5.5 hours with 20 ml. of concentrated hydrochloric acid. The solution is vacuum-distilled to dryness, water is added, and the distillation is repeated. A solution of the residue in 50 ml. of water is passed slowly through a column of anion exchange resin (Note 5). The combined effluent and washings (Note 6) are evaporated to dryness under reduced pressure. The residue is decolorized in ethanol solution with Norit, and the filtrate ie evaporated to dryness. The residue, thoroughly dried over phosphorus pentoxide, is dissolved in warm 95% alcohol, to which acetone is added to the point of incipient turbidity. The solution is stored overnight in a refrigerator, and the product is collected. A second crop is obtained by evaporating the mother liquor to small volume and diluting with 5 ml. of absolute alcohol. The combined yield is 0.55 g., 59% based on reacted cyanide. Purification is accomplished by trituration with a little cold absolute ethanol, followed by treatment with 25 ml. of acetone and filtration. The product, m.p. 155–156°, is halogen-free and gives only one ninhydrin-reactive, radioactive spot with paper chromatography (Note 7).

B. Notes

1. Directions are given by Rothstein for preparing the starting material (b.p. 94–95° at 18 mm.) by reacting γ-butyrolactone with hydrobromic acid, followed by esterification[1] of the 4-bromobutyric acid.

2. Argentimetric titration of the contents of the trap indicates that 36% of the cyanide is recovered unreacted.

3. Trial experiments have shown that the optimal conditions consist of refluxing the potassium cyanide with up to 30% excess of the ester in 70% alcohol for 12–16 hours. The yield is 61% based on the cyanide

added, as determined by hydrolyzing the oily ethyl 4-cyanobutyrate to glutaric acid; see ethyl 2-acetamido-2-carbethoxy-5-cyano-C^{14}-valerate.

4. The apparatus consists of a Parr hydrogenator.

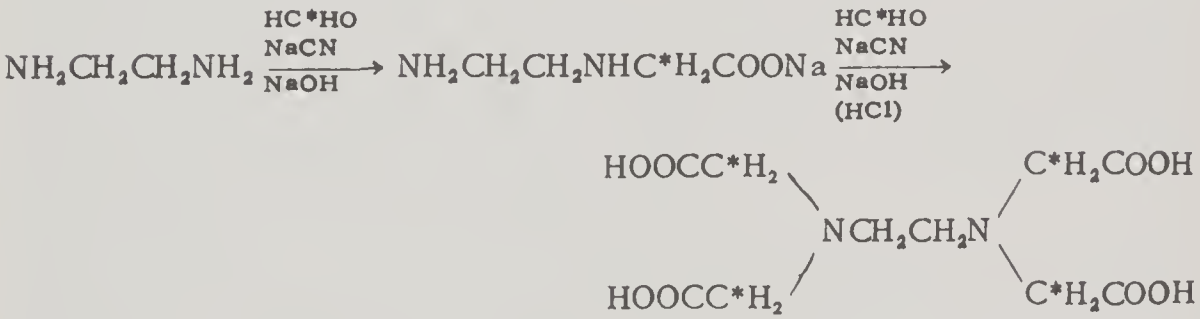
5. Amberlite IR-4 in the carbonate form.

6. About 200 ml. of water is required to give a ninhydrin-negative, non-adioactive wash liquor.

7. The spots coincide with those obtained with an authentic sample developed in collidine-lutidine-water and phenol-water.

[1]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 246.

(ETHYLENEDINITRILO)TETRAKIS(ACETIC-2-C^{14}) ACID
[Ethylenediaminetetra(acetic-2-C^{14}) Acid]
(C_4^{14}-Versene)



A. Murray III and A. R. Ronzio, J. Am. Pharm. Assoc., Sci. Ed., *41*, 596 (1952).

A. Procedure (Note 1)

The apparatus consists of a 100-ml. round-bottomed, two-necked flask equipped with a reflux condenser, a magnetic stirrer and a 15-ml., pressure-compensating addition tube (Note 2), which feeds reagent through a fine drip-tip into a nitrogen stream emerging beneath the surface of the reaction mixture.

To a stirred, ice-cooled mixture of 0.3596 g. (5.97 mmoles) of ethylenediamine (freshly distilled from sodium), 7 ml. of water, 0.30 g. of sodium hydroxide and 17.9 mmoles of sodium cyanide is added, at a uniform rate over 1 hour, a 19.5 ml. aliquot (7.48 mmoles) of aqueous formaldehyde-C^{14} solution (Note 3). The mixture is stirred overnight at room temperature, then refluxed for 10 hours and concentrated to 10–15 ml. by distillation (Note 4). To the boiling mixture is added a 1-ml. portion of a solution containing 26.9 mmoles of sodium cyanide, 0.30 g. of sodium hydroxide and water to 7.0 ml. The remainder of the formaldehyde-C^{14} solution is added at the original rate, while the reaction mixture is distilled at such a rate as to maintain the small volume (Note 5), 1 ml. of the cyanide solution being added after the addition of each 5 ml. of formaldehyde-C^{14} so-

lution. The reaction is completed in the same manner with: a) a solution of 23.9 mmoles of sodium cyanide, 0.15 g. of sodium hydroxide and water to 8 ml. (2 ml. per addition), and b) 11.94 mmoles of nonisotopic formaldehyde in 20 ml. of water (Note 6). Complete hydrolysis of the mixture is assured by refluxing until no further ammonia is evolved. The mixture is neutralized with concentrated hydrochloric acid, boiled for a few minutes and cooled, and the precipitated silicic acid (Note 7) is filtered off and thoroughly washed with water. The combined filtrate and washings are concentrated to 10–15 ml., refiltered, adjusted to pH 1.20 with concentrated hydrochloric acid and stored at 0° for at least 24 hours. The product is collected on a chilled funnel, washed chloride-free and dried for 3 hours at 100°; yield 1.2695 g. (72.8% based on ethylenediamine), m.p. 243° (dec.) (Note 8). The best recrystallization medium is water (Note 9). The specific activity is in agreement with the expected value.

B. Notes

1. The procedure is a modification of the methods described by Smith[1] and Bersworth[2] for the carboxymethylation of ethylenediamine, considerable alteration being necessitated as the scale was reduced to the 3-mmole level. The reaction between ethylenediamine and sodium chloroacetate is unsatisfactory at the semimicro scale, because of low yield and considerable by-product formation.

2. To assure uniform addition of reagent, the usual lubricated stopcock is replaced by a lapped-glass needle valve, which is controlled by a remote fine-thread screw.

3. The stock solution contains 20.9 mmoles/54.5 ml.

4. The ethylenediamine is fixed in a nonvolatile form, prior to the distillation, by reaction with 1.25 equivalents of formaldehyde-C^{14} and excess cyanide in the cold and in the absence of ammonia (until heat is applied).

5. Satisfactory ammonia removal, which is all-important in the small-scale preparation, is accomplished by active distillation from a minimal volume of solvent in a stream of nitrogen. No ethylenediamine is lost by entrainment in the gas stream.

6. The combined distillate contains very little activity.

7. The silicic acid results from decomposition of the glass in the presence of alkali.

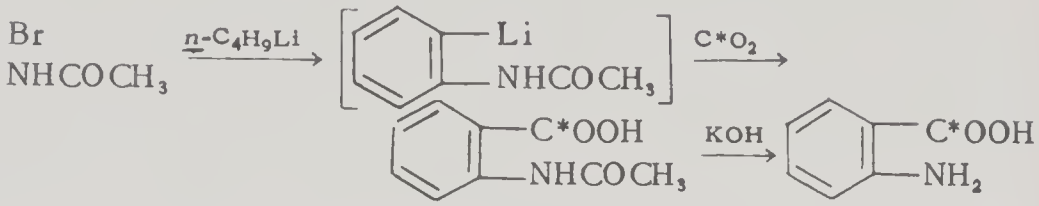
8. The yield in three trial runs was 73.2–80.7%. The material cannot be sublimed, and no residue is left following ignition.

9. The water solubility is 6.7 mg./ml. at 92° and 0.53 mg./ml. at 30°.

[1]R. Smith, J. L. Bullock, F. C. Bersworth and A. E. Martell, J. Org. Chem., *14*, 355 (1949).

[2]F. C. Bersworth, U. S. 2,387,735 and 2,407,645; Chem. Abstracts, *40*, 1171 (1946); *41*, 776 (1947).

ANTHRANILIC-C^{14} ACID
(o-Aminobenzoic-C^{14} Acid)



A. Murray III and A. R. Ronzio, Atomic Energy Commission Report AECU-85; Nuc. Sci. Abstracts, *2*, 908 (1949).

A. Procedure (Note 1)

To a fresh solution of 3.6 mmoles of butyllithium in 2.83 ml. of absolute ether, maintained at −30° under purified nitrogen, is added a solution of 0.428 g. (2.0 mmoles) of 2′-bromoacetanilide (Note 2) in 15 ml. of absolute ether over a period of 2 minutes. The colorless solution is stirred for 15 minutes at −30° (Note 3), then is cooled to −78°, and the flask is evacuated to 0.1 mm. Carbonation is achieved with stirring at −78°, by admitting the carbon-C^{14} dioxide prepared from 0.197 g. (1 mmole) of barium carbonate-C^{14}. The mixture alternately is frozen with liquid nitrogen and thawed with a Dry Ice-bath several times to complete the absorption. Nitrogen is admitted to the system, and the mixture is hydrolyzed with 9 ml. of water containing 0.5 ml. of concentrated hydrochloric acid. Unreacted carbon-C^{14} dioxide, and any released by hydrolysis, is distilled into a trap cooled with liquid nitrogen, and collected for recovery in an alkali scrubber by sweeping the system with a slow stream of nitrogen. The aqueous solution is made strongly basic with potassium hydroxide and continuously extracted with ether for 5 hours. The aqueous solution (50 ml.) is freed from dissolved ether by warming, then treated with 20 ml. of alcohol (Note 4) and refluxed for 40 hours to hydrolyze the *N*-acetylanthranilic-C^{14} acid. The alkaline mixture is extracted with ether for 3 hours; then the aqueous solution is adjusted to pH 3.0 with hydrochloric acid and exhaustively extracted with ether for 24–48 hours (Note 5). The extract of crude product is evaporated and dried in a dessicator over phosphorus pentoxide; yield 0.0712 g. (52%). Purification is carried out by sublimation at 90–100° at 0.1 mm. (Note 6). The sublimate is dissolved in ether and treated with carbon, and the filtrate is evaporated slowly to form colorless needles; yield 0.0552 g. m.p. 140–142° (Note 7).

B. Notes

1. For a similar preparation see *p*-aminobenzoic-C^{14} acid.

2. 2′-Bromoacetanilide is dried over phosphorus pentoxide. Blocking of the amine group reduces the number of active hydrogens and conserves

isotope. Trial experiments with *o*-bromoaniline and the halogen-metal interconversion reaction, in both ethyl ether and petroleum ether, gave unsatisfactory yields of anthranilic acid, following carbonation with carbon dioxide as the limiting reagent. Gilman and Stuckwisch[1] have reported yields of 40% based on *o*-bromoaniline, carbonating with an excess of Dry Ice, presumably, as is the usual practice.

3. A greenish-yellow color slowly develops.

4. The alcohol controls foaming.

5. Complete removal of anthranilic acid from the aqueous solution is indicated by the loss of fluorescence under ultraviolet light.

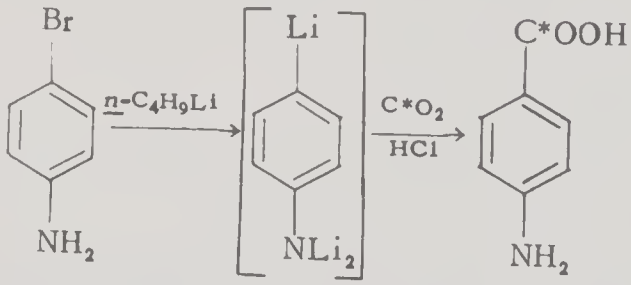
6. The sublimation is continued until ether added to the residue no longer shows fluorescence.

7. A brown color, which developed during a 4-month storage period in a closed tube at room temperature, was removed readily by resublimation. Storage under refrigeration is recommended. The self-radiolysis of compounds of high specific activity may be minimized by storage in dilute solution, or as an intimate mixture of finely divided particles (<< 0.1 mm.) with a large amount of an inert finely divided solid, such as glass.[2]

[1] *Organic Reactions*, Vol. VI, Wiley, New York, 1951, p. 358.
[2] R. M. Lemmon, Nucleonics, *11*, (10), 44 (1953).

p-AMINOBENZOIC-C^{14} ACID

METHOD I



A. Murray III, W. W. Foreman and W. Langham, J. Am. Chem. Soc., 70, 1037 (1948); Science, *106*, 277 (1947).

A. Procedure (Note 1)

The apparatus is a modification of that described for the gaseous carbonation of Grignard reagents; see Figure II, 11. The carbonation flask is equipped with a pressure-equalizing addition tube and a siphon connected, through a stopcock, to an evacuated reservoir-flask. The addition tube is surmounted by a second pressure-equalizing addition tube containing wash ether and is isolated, by stopcocks, from the lower tube and from the atmosphere. A mercury-filled U-tube serves as a convenient pressure indicator for the upper addition tube. The manifold is equipped with a

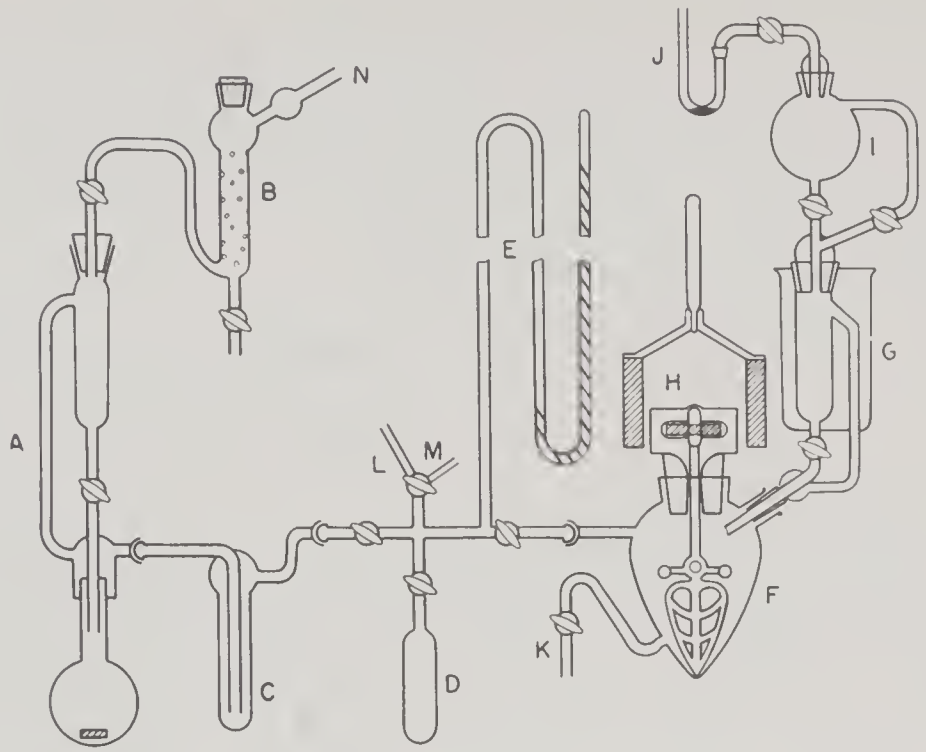


Fig. II, 11. Apparatus for the preparation and carbonation of organolithium compounds (A. Murray III, W. W. Foreman and W. Langham). A, carbon dioxide generator; B, alkali bubbler trap; C, Dry Ice-trap; D, carbon dioxide reservoir; E, mercury manometer; F, reaction flask; G, reagent addition tube with cooling bath; H, magnetic stirrer; I, reservoir for wash liquor; J, mercury trap; K, drain to low-temperature receiver; L, outlet to vacuum; M, nitrogen inlet; N, Ascarite.

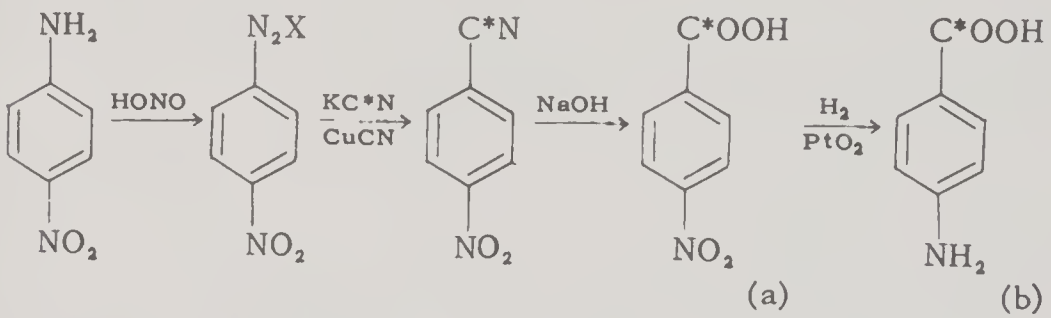
bulb for storage of frozen carbon-C^{14} dioxide. The generator is connected, by stopcock, to an absorption tower containing carbonate-free sodium hydroxide[1] for recovery of carbon-C^{14} dioxide, and is protected from the atmosphere by a guard tube (Note 2).

Carbon-C^{14} dioxide, prepared by adding outgassed concentrated sulfuric acid slowly to dry barium carbonate-C^{14} *in vacuo*, is distilled through a trap cooled with Dry Ice-acetone and condensed in a storage bulb cooled with liquid nitrogen (Note 3). Any noncondensable gas is pumped off.

The reaction flask is evacuated (0.1 μ), flamed, swept with dry nitrogen and charged with 29 ml. of an ethereal solution of 23.8 mmoles of freshly prepared butyllithium, which is delivered by a syringe-operated pipette, previously flushed with nitrogen (Note 4). To the stirred solution, cooled with an ice-bath, is added during 5 minutes a solution of 4.74 mmoles of *p*-bromoaniline (vacuum-dried) in 8 ml. of absolute ether. Following the appearance of a bright yellow precipitate (Note 5), the mixture is stirred at room temperature for 1.5 hours. The precipitate is allowed to settle and is washed 4–5 times with absolute ether by decantation, the supernatant liquors being siphoned off under nitrogen into the evacuated, Dry Ice-cooled reservoir flask (Note 6). The stirred suspension is cooled to $-80°$, pumped down to the vapor pressure of ether, and carbontated with

1.029 mmoles of carbon-C^{14} dioxide. When the pressure becomes constant, the mixture is alternately frozen with liquid nitrogen and thawed several times to complete the gas absorption. Any unreacted carbon-C^{14} dioxide is distilled back into the storage bulb with liquid nitrogen. The system is filled with nitrogen, and the addition tube is charged quickly with 8 ml. of 6 *N* hydrochloric acid. The cold, stirred reaction mixture is hydrolyzed slowly, and any liberated carbon-C^{14} dioxide is condensed into the storage bulb. The system is swept for about 1 hour with a slow stream of nitrogen, which is bubbled through the absorption tower for recovery of carbon-C^{14} dioxide (Note 7). The strongly acidic solution is extracted continuously with ether for 8 hours, then made basic with potassium hydroxide, and the extraction is continued for 8 hours. The aqueous solution is adjusted to pH 3, and the product is collected by extraction with ether for 16 hours (Note 8). The yield of crude product is 32.8% based on barium carbonate (Note 9), or 48.2% based on unrecovered carbon-C^{14} dioxide. Purification is achieved by vacuum sublimation and recrystallization from water; yield 0.035 g. of colorless needles, m.p. 184–185°.

METHOD II



A. Wacker, H. Grisebach, A. Trebst and F. Weygand, Angew. Chem., 66, 326 (1954).

A. Procedure

(a) p-*Nitrobenzoic-C^{14} Acid.* The diazonium salt solution prepared from 0.2 g. (1.5 mmoles) of *p*-nitroaniline is neutralized with calcium carbonate, filtered, and added to a solution of 0.065 g. (1 mmole) of potassium cyanide-C^{14} and 0.030 g. of cuprous cyanide in 2 ml. of water at 40°. The mixture is heated at 40° for 1 hour; then the *p*-nitrobenzonitrile-C^{14} is hydrolyzed by heating at 100° for 1 hour with 2 ml. of sodium hydroxide solution. Purification is accomplished by vacuum sublimation; yield 0.060 g., 36% based on cyanide (see 4-nitro-o-anisonitrile-C^{14}).

(b) p-*Aminobenzoic-C^{14} Acid.* The *p*-nitrobenzoic-C^{14} acid is hydrogenated as described for the preparation of 4-aminosalicylic-C^{14} acid. The product is purified by vacuum sublimation; yield 0.060 g. (36% based on potassium cyanide-C^{14}), m.p. 189°

B. Notes

1. The procedure is an adaptation of the method of Gilman and Stuckwisch.[2] The rapid halogen-metal interconversion reaction[3] between an alkyllithium and an aryl halide is uniquely suited to the preparation of organolithium compounds, which are readily carbonated, forming carboxylic acids having additional functional groups.

2. Stopcocks are lubricated with silicone grease, and a plug of glass wool is employed to prevent barium carbonate dust from being carried out of the generator.

3. The evolution of gas is completed by stirring (magnetically) the sulfuric acid mixture (15–20 ml. per gram of barium carbonate) and heating with a soft flame to dissolve all solids.

4. The butyllithium is prepared in the manner described by Gilman *et al.*,[4,3] assayed immediately before use, by the differential titration procedure of Gilman and Haubein,[5,3] and used within 48 hours. Concentrations of the order of 1–2 *N* may be used satisfactorily. The reagent is stored at the temperature of an ice-salt bath, to clarify the solution, and the clear supernatant solution is pipetted off as required. The reagent slowly cleaves ether, giving rise to lithium ethoxide, which is reactive towards carbon dioxide. The reagent is discarded as unsuited for isotopic work, when the second titration of the assay procedure[5,3] indicates the presence of appreciable alkali (other than butyllithium).

5. The precipitate begins to form in 20–90 minutes, depending upon the age of the butyllithium. The yield is unaffected by varying the reaction time from 1 to 3 hours.

6. The dry *p*-aminophenyllithium derivative is explosive on contact with air. The washing removes the excess of butyllithium.

7. Of the initial barium carbonate-C^{14}, 32.5% is recovered from the scrubber by precipitation with barium chloride according to the procedure described by Calvin.[6]

8. Ether extraction is superior to the formation of the copper and silver salts for isolating the product at the 1-mmole level.

9. Trial runs consistently have given yields of 31.3–38.5%. Attempts to improve the yield by blocking the amino group by acetylation (see anthranilic-C^{14} acid) were unsuccessful.

C. Other Preparations

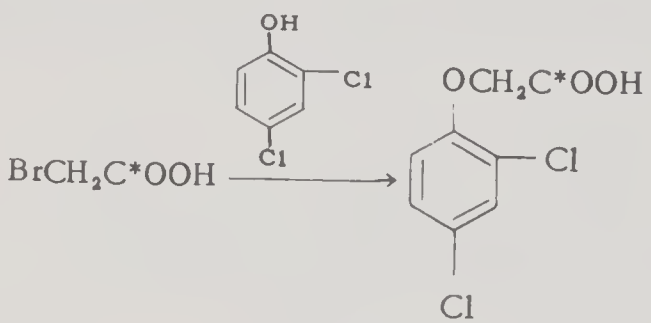
A preparation of C^{14}-*p*-aminobenzoic acid has been reported by Markova.[7]

A preparation of 3-hydroxyanthranilic-C^{14} acid has been outlined by Ciereszko.[8] A mixture of 2.7 g. (10 mmoles) of 3-iodo-2-nitroanisole[8] and 0.9 g. (10 mmoles) of dry cuprous cyanide (–C^{14}) was heated at 180° for 2 hours. By extraction with 25 ml. of boiling benzene and precipitation with 50 ml. of ligroin was obtained 1.22 g. of crude 2-nitro-*m*-anisonitrile

($-C^{14}$) (yield 70%, m.p. 117–122°). Recrystallization from benzene-ligroin raised the melting point to 122°. Hydrolysis by refluxing 0.9 g. (5 mmoles) of the nitrile with 16 ml. of water and 4 ml. of alcohol for 1.5 hours gave a quantitative yield of 2-nitro-*m*-anisic ($-C^{14}$) acid, m.p. 257° from alcohol. Hydrogenation over palladium and demethylation with phosphorus and hydriodic acid by the procedure of Nyc[9] gave the product.

[1]W. G. Dauben, J. C. Reid and P. E. Yankwich, Anal. Chem., *19*, 828 (1947).
[2]H. Gilman and C. G. Stuckwisch, J. Am. Chem. Soc., *63*, 2844 (1941).
[3]R. G. Jones and H. Gilman, *Organic Reactions*, Vol. VI, Wiley, New York, 1951, Chapter 7.
[4]H. Gilman, J. A. Beel, C. G. Brannen, M. W. Bullock, G. E. Dunn and L. S. Miller, J. Am. Chem. Soc., *71*, 1499 (1949).
[5]H. Gilman and A. H. Haubein, J. Am. Chem. Soc., *66*, 1515 (1944).
[6]M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 83.
[7]Y. V. Markova, L. N. Zenkova and M. N. Shchukina, Zhur. Obshcheĭ Khim., *25*, 1383 (1955); Nuc. Sci. Abstracts, *9*, 7315 (1955).
[8]L. S. Ciereszko and L. V. Hankes, J. Am. Chem. Soc., *76*, 2500 (1954).
[9]J. F. Nyc and H. K. Mitchell, J. Am. Chem. Soc., *70*, 1847 (1948).

(2,4-DICHLOROPHENOXY)ACETIC-1-C^{14} ACID
(C_1^{14}-2,4-D)



R. W. Holley, F. P. Boyle and D. B. Hand, Arch. Biochem. *27*, 143 (1950).

A. Procedure (Note 1)

A mixture of 0.400 g. of 2,4-dichlorophenol, 0.112 g. of sodium hydroxide, 0.5 ml. of water and the crude bromoacetic-1-C^{14} acid prepared from 0.4 mmole of sodium acetate-1-C^{14} (Note 2) is heated for 6 hours at 100°. The solution is diluted with water, then saturated with carbon dioxide and extracted repeatedly with ether. The extract is washed with 1 ml. of water and evaporated, and the residue is dried under vacuum; yield 0.077 g. The product is recrystallized by diluting 0.8 ml. of benzene solution with 4 ml. of petroleum ether; yield 0.070 g. (Note 3).

B. Notes

1. The procedure is adapted from the method of Pokorny.[3]

2. Fields[1] employed a fifteen-fold excess of 2,4-dichlorophenol in sodium hydroxide solution; yield 0.210 g., 95%, m.p. 138.5–139.5°

3. The product from a similar trial run melted at 138–139° (cor.), neutralization equivalent 223 (calc. 221).

C. Other Preparations

In a similar manner, starting with bromoacetic-2-C^{14} acid, is prepared[1,2] (2,4-dichlorophenoxy)acetic-2-C^{14} acid.

The preparation of (2,4-dichlorophenoxy)acetic-1-C^{14} acid (yield 60–80%), by a similar alkaline condensation employing chloroacetic-1-C^{14} acid, has been reported by Mahler.[4]

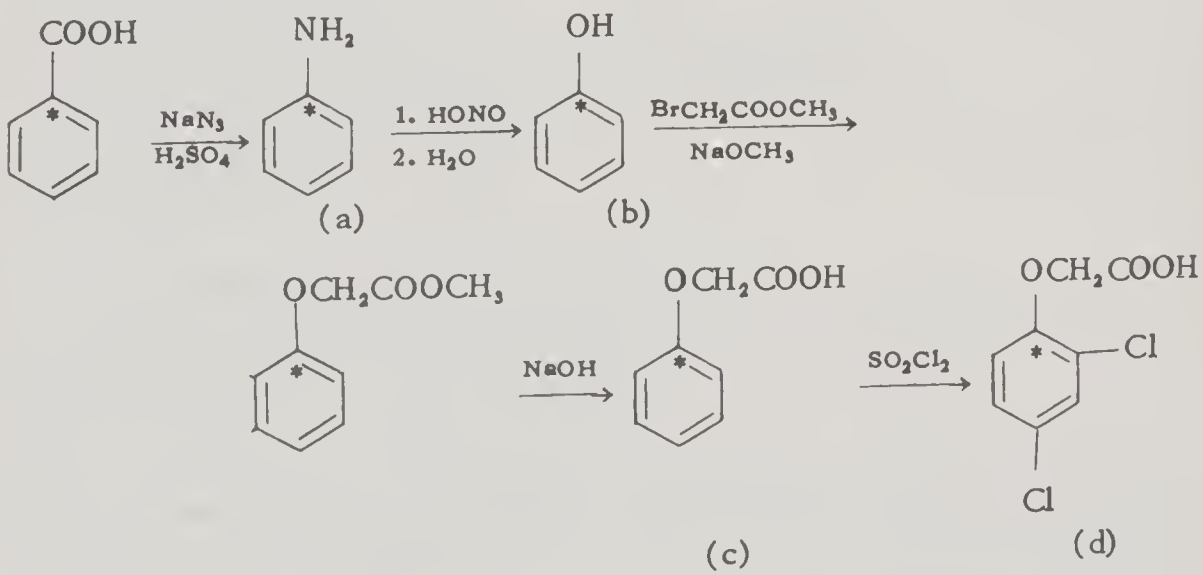
[1]M. Fields, S. Rothchild and M. A. Leaffer, J. Am. Chem. Soc., 74, 2434 (1952).

[2]S. C. Fang, E. G. Jaworski, A. V. Logan, V. H. Freed and J. S. Butts, Arch. Biochem., 32, 249 (1951).

[3]R. Pokorny, J. Am. Chem. Soc., 63, 1768 (1941); Wei-Kuang Cheng and Chin-Hsu Liu, Biochem. Bull. (China) No. 59, 1 (1946); Chem. Abstracts, 40, 2441 (1946).

[4]H. R. Mahler, R. J. Speer and A. Roberts, Science, *110*, 562 (1949).

(2,4-DICHLOROPHENOXY-1-C^{14})ACETIC ACID (C_1^{14}-2,4-D)



M. Fields, S. Rothchild and M. A. Leaffer, J. Am. Chem. Soc., 74, 2435 (1952).

A. Procedure

(a) *Aniline-1-C^{14} Hydrochloride.* To a rapidly stirred mixture of 12.2 g. of benzoic-1-C^{14} acid, 80 ml. of chloroform and 40 ml. of 100% sulfuric

acid (Note 1) maintained at 40° is added 10 g. of sodium azide over a period of 50 minutes. When the nitrogen evolution has ceased, the reaction mixture is diluted with water, made alkaline and extracted with ether. Treatment with dry hydrogen chloride precipitates the product; yield 11.8 g. (91%), m.p. 194.5°.

(b) *Phenol-1-C*^{14}. Aniline-1-C^{14} hydrochloride (13.00 g.) is diazotized, and the diazonium salt is hydrolyzed; yield 7.04 g. (75%), b.p. 95° (25 mm.). See Other Preparations.

(c) *Phenoxy-1-C*^{14}*-acetic Acid.* Phenol-1-C^{14} is converted to methyl phenoxy-1-C^{14}-acetate by reaction with methyl bromoacetate in the presence of sodium methoxide, after the method of Fusco and Mazzucchi[1] (Note 2). The ester is hydrolyzed in dilute sodium hydroxide, and the product, obtained upon acidification, melts at 97–98° (90% yield).

(d) (*2,4-Dichlorophenoxy-1-C*^{14})*acetic Acid.* The temperature of a mixture of 1.74 g. of phenoxy-1-C^{14}-acetic acid, 0.39 g. of sulfuryl chloride and 7 ml. of glacial acetic acid is raised slowly from 0° to 75° and held there until gas evolution ceases (Note 3). The mixture is diluted with water, and the precipitate is recrystallized from benzene; yield 1.87 g. (74%), m.p. 137–138°.

B. Notes

1. The concentration of sulfuric acid in the Schmidt reaction determines the yield of aniline hydrochloride. The following figures represent percentages, respectively, of sulfuric acid, aniline hydrochloride, benzoic acid recovered and aniline hydrochloride corrected: a) 100, 91, 0, 91; b) 98, 71, 13, 89; c) 96, 60–65, 20–35, 75–87. With 75% acid the yield of aniline[2] is 15%. Increasing the quantity of 96% sulfuric acid or of sodium azide has no appreciable effect on the yield. Briggs and Lyttleton[3] have obtained a 69% yield of aniline, employing concentrated sulfuric acid and trichloroethylene as solvent, while with dry ether[2] the yield was 24%. The use of dilute oleum invariably gives quantitative yields of sulfanilic acid.[7]

2 The yield of ethyl ester made from chloroacetate[1] is 87%.

3. In the absence of solvent the 4-chloro compound is formed, while in boiling carbon tetrachloride no reaction takes place. Haskelberg[4] has reported an 82% yield of 2,4-D, at the 1-mole scale, by halogenation with chlorine gas in acetic acid solution. Stopping the reaction at the dichloro compound might be troublesome on a very small scale.[5] Chlorination with alkaline hypochlorite has given the desired product in variable yields of less than 59%[5] up to 75%.[6]

C. Other Preparations

The preparation of aniline-$C^{14}_{1/4}$ hydrochloride by the described procedure from benzoic-$C^{14}_{1/8}$ acid has been reported by Kilner;[7] yield 95% at the 2 to 15-mmole level.

Phenol-$C^{14}_{1/4}$ is prepared[7] at the 15-mmole scale in better than 95% yield based on benzoic-1,2,3,4-$C^{14}_{1/4}$ acid, by a modification of the described procedure. The diluted diazonium solution is added gradually to aqueous sulfuric acid at 200°, the product being swept out in a very rapid current of steam. A degradation of phenol is outlined.[7]

[1]R. Fusco and F. Mazzucchi, Gazz. chim. ital., *71*, 406 (1941); through Chem. Abstracts, 37, 121 (1943).

[2]L. H. Briggs, G. C. DeAth and S. R. Ellis, J. Chem. Soc., *1942*, 61.

[3]L. H. Briggs and J. W. Lyttleton, J. Chem. Soc., *1943*, 421.

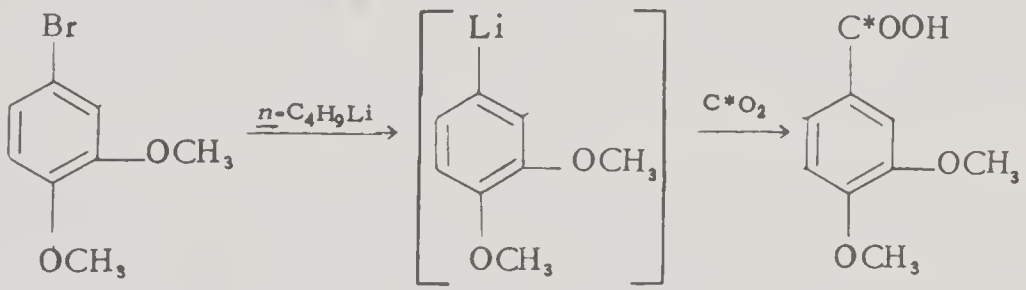
[4]L. Haskelberg, J. Org. Chem., *12*, 426 (1947).

[5]M. Fields, S. Rothchild and M. A. Leaffer, J. Am. Chem. Soc., *74*, 2435 (1952).

[6]C. Y. Hopkins and M. J. Chisholm, Can. J. Research, *24B*, 208 (1946).

[7]A. E. H. Kilner, H. S. Turner and R. J. Warne, *Radioisotope Conference*, 1954, Vol. II, Academic Press, New York, 1954, p. 23.

VERATRIC-C^{14} ACID
(3,4-Dimethoxybenzoic-C^{14} Acid)



M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 183.

A. Procedure (Note 1)

Into the nitrogen-filled reaction flask of a Grignard carbonation apparatus (Note 2) is pipetted 20.0 ml. of a pentane solution containing 12.6 mmoles of butyllithium (Note 3). The solution is concentrated to approximately 1 ml. by vacuum distillation, then is chilled, diluted with about 20 ml. of absolute ether by distillation, mixed, and frozen with liquid nitrogen. Additional ether (30 ml.) is distilled into the flask and frozen. The system again is filled with nitrogen, and 4.08 g. (18.8 mmoles) of 4-bromoveratrole in a little ether is added and frozen. The system is evacuated and isolated from the pump; then the stratified mixture is thawed with a bath at −60° and stirred for 15 minutes after the precipitation of veratryllithium. Carbonation is effected as quickly as possible at −60° with the carbon-C^{14} dioxide from 1.25 g. (6.35 mmoles) of barium carbonate-C^{14} (Note 4). Nitrogen is admitted, and the mixture is hydrolyzed with 10 ml. of concentrated hydrochloric acid. The product is extracted into ether and taken into sodium hydroxide solution, which is then warmed

decolorized with carbon, filtered, and acidified to precipitate the product; yield 1.03 g. (89.6% based on barium carbonate), m.p. 176–178° (Note 5).

B. Notes

1. The organolithium compound, rather than the usual Grignard reagent, is carbonated because of the difficulty in preparing the latter. Also see nicotinic-C^{14} acid. Use of this procedure has been reported by Swan.[1]

2. See benzoic-C^{14} acid.

3. The reagent is prepared by refluxing overnight, under pure nitrogen, a mixture of 2.5 g. of shiny lithium chips, 10 g. of butyl chloride and 20 ml. of dry pentane. The solution is diluted with 80 ml. of pentane and filtered under nitrogen. An aliquot is assayed by hydrolysis with standard acid, the excess of which is determined by titration with standard alkali.

4. The reaction requires about 7 minutes.

5. The neutralization equivalent is 184 (calc'd. 182), and the product tests halogen-free.

C. Other Preparations

In a similar manner from 1-iodo-3,4,5-trimethoxybenzene has been prepared[2] 3,4,5-trimethoxybenzoic-C^{14} acid (yield 74%) which was converted to syringic-C^{14} acid, (4-hydroxy-3,5-dimethoxybenzoic-C^{14} acid), by demethylation with concentrated sulfuric acid[3] for six hours at 40° (yield 75%).

The preparation of 4-benzyloxy-*m*-anisic-C^{14} acid by the bromine-metal exchange reaction has been reported by Brown[2] and described by Kratzl[4] in detail with a diagram of the system used; yield 71.5%, m.p. 155–160°. Recrystallization from alcohol raised the melting point to 168–169° (70% recovery). Debenzylation by boiling with 6 *N* hydrochloric acid gave vanillic-C^{14} acid, (4-hydroxy-3-methoxybenzoic-C^{14} acid), (71% yield) which was demethylated to protocatechuic-C^{14} acid, (3,5-dihydroxybenzoic-C^{14} acid), (77% yield) by refluxing for 5 hours with 45% hydriodic acid—glacial acetic acid (3 : 10 by vol.).[2]

p-Anisic-C^{14} acid has been cleaved by refluxing with aluminum bromide in benzene, forming *p*-hydroxybenzoic-C^{14} acid (m.p. 204°) which is purified by carbon disulfide extraction, in which solvent it is insoluble.[5] See *p*-anisic-C^{14} acid.

The similar preparation of 4-benzyloxy-3,5-dimethoxybenzoic-C^{14} acid from the iodo compound has been reported by Freudenberg.[6] The starting halide was prepared[7] by the Sandmeyer reaction from the amine, which was obtained by the action of hypochlorite on the amide.

[1]G. A. Swan and D. Wright, J. Chem. Soc., *1954*, 381.
[2]S. A. Brown and A. C. Neish, Can. J. Biochem. and Physiol., 33, 948 (1955).

[3]M. T. Bogert and B. B. Coyne, J. Am. Chem. Soc., *51*, 569 (1929).
[4]K. Kratzl and G. Billek, Holzforschung, *7*, 66 (1953).
[5]L. S. Ciereszko and J. G. Burr, Jr., J. Am. Chem. Soc., *74*, 5431 (1952).
[6]K. Freudenberg and M. Reichert, Naturwiss., *41*, 229 (1954).
[7]K. Freudenberg and M. Reichert, Ber., *87*, 1834 (1954).

C_1^{14}-BROMOACETIC ACID

METHOD I

$$C^*H_3COOK \xrightarrow[H_3PO_4]{} C^*H_3COOH \xrightarrow[(CH_3CO)_2O]{Br_2} C^*H_2BrCOOH \xrightarrow[HBr]{C_2H_5OH} C^*H_2BrCOOC_2H_5$$

(a) (b)

F. Kögl, P. Emmelot and D. H. W. den Boer, Ann., *589*, 1 (1954).

A. Procedure

(a) *Bromoacetic-2-C^{14} Acid* (Note 1). Thoroughly dried potassium acetate-2-C^{14} (prepared from 16.5 mmoles of barium carbonate-C^{14}) is treated with phosphoric acid saturated with phosphoric anhydride. The acetic-2-C^{14} acid is collected by distillation on the vacuum line, then mixed with 1 ml. of acetic anhydride, heated for 1 hour at 100° under reflux, and finally brominated by the dropwise addition of 2 g. of purified bromine (Note 2). Hydrogen bromide is evolved, and the product is formed in practically quantitative yield (Note 3).

(b) *Ethyl Bromoacetate-2-C^{14}*. To the crude bromoacetic-2-C^{14} acid saturated with hydrogen bromide is added an excess of absolute ethanol (5 ml.), and the mixture is heated for 2 hours on a steam-bath. The mixture is neutralized with cold sodium carbonate solution, and the product is extracted into ether (Note 4).

METHOD II

$$CH_3C^*OONa \xrightarrow[\substack{CH_3COCl \\ P,HCl}]{Br_2} BrCH_2C^*OOH$$

M. Fields, S. Rothchild and M. A. Leaffer, J. Am. Chem. Soc., *74*, 2435 (1952).

A. Procedure

Bromoacetic-1-C^{14} Acid (Note 5). Into a 25-ml. flask, containing 0.082 g. (1.0 mmole) of sodium acetate-1-C^{14} and 1.5 mg. of red phosphorus, is vacuum-transferred, with liquid nitrogen cooling, 0.0375 g. (1.0 mmole) of anhydrous hydrogen chloride, 0.0079 g. (1 mmole) of freshly distilled acetyl chloride and 0.192 g. (1.2 mmoles) of bromine (Note 6).

The flask is sealed under vacuum and heated in a boiling water-bath until the bromine color has largely disappeared (1–5 hours). The product is dissolved in 1 ml. of water; yield 75–80% (Note 7).

Bromoacetic-2-C^{14} acid is prepared in the same manner from sodium acetate-2-C^{14}.

METHOD III

$$CH_3C^*OOH \xrightarrow[C_5H_5N,\ Ac_2O]{Br_2} BrCH_2C^*OOH$$

L. I. Gidez and M. L. Karnovsky, J. Am. Chem. Soc., *74*, 2413 (1952).

A. Procedure

Acetic-1-C^{14} or -2-C^{14} acid is brominated in 84% yield by an adaptation of the method of Natelson and Gottfried,[1] modified by employing 50% more bromine (Note 8).

METHOD IV

$$C^*H_3COOH \xrightarrow[Ac_2O]{Br_2,P} BrC^*H_2COBr \xrightarrow{H_2O} BrC^*H_2COOH$$

G. A. Ropp, J. Am. Chem. Soc., *72*, 4459 (1950).

A. Procedure (Note 9)

Bromoacetic-2-C^{14} Acid. To 0.590 g. (9.83 mmoles) of acetic-2-C^{14} acid are added 2 drops of acetic anhydride (equivalent to 0.63 mmole of acetic acid), 3 mg. of red phosphorus and 0.6 ml. of bromine. The mixture is heated for 1 hour at 125–135°; then hydrogen bromide and excess bromine are removed by bubbling in dry carbon dioxide at 50°. The yield of crude product is 1.393 g. of clear oil (102%), purity 83% (Note 10).

METHOD V

$$CH_3C^*OBr \xrightarrow{Br_2} BrCH_2C^*OBr \xrightarrow{H_2O} BrCH_2C^*OOH$$

K. Bloch, J. Biol. Chem., *179*, 1245 (1949).

A. Procedure (Note 11)

Bromoacetic-1-C^{14} Acid. To acetyl-1-C^{14} bromide, in an ice-cooled flask, is added slowly 1.1 molar amounts of bromine. The mixture is refluxed on a steam-bath for 1.5 hours, then freed of excess bromine and hydrogen bromide by a stream of nitrogen. The bromoacetyl-1-C^{14} bromide

is hydrolyzed by the dropwise addition of cold water with ice-bath cooling.

METHOD VI

$$C^*H_3C^*OOH \xrightarrow[P]{Br_2} BrC^*H_2C^*OBr \xrightarrow{C_2H_5OH} BrC^*H_2C^*OOC_2H_5$$

B. Schepartz and S. Gurin, J. Biol. Chem., *180*, 663 (1949); W. Sakami, W. E. Evans and S. Gurin, J. Am. Chem. Soc., *69*, 1110 (1947).

A. Procedure

Ethyl Bromoacetate-C_2^{14}. Sodium acetate-C_2^{14} (6.0 g.) is converted to 10.5 g. of ethyl bromoacetate-C_2^{14}, b.p. 172° (86% yield), by the method of Auwers and Bernhardi,[2] which follows.

To a mixture of 20 g. of acetic acid and 3.5 g. (3.4 g., theory) of dry red phosphorus is added gradually 230 g. of dry bromine, with suitable cooling if necessary. The mixture is heated under reflux for 5 hours on a water-bath, with recovery of considerable bromine, then treated slowly with 2–3 times the calculated amount of absolute alcohol. The mixture is diluted with water, and the product is extracted into ether solution, which is washed with sodium bicarbonate solution, dried and fractionally distilled at 159° (Note 12).

Bromoacetic-1-C^{14} acid[3] and bromoacetic-2-C^{14} acid[4] (85–90%) are prepared by this procedure.

B. Notes

1. The procedure is an adaptation of that of Shaw,[5] the acetic anhydride serving as carrier for bromine.

2. The bromine is dried with sulfuric acid and distilled to remove traces of the acid which otherwise check the reaction.

3. At the usual laboratory level the yield is 96% (b.p. 203–207°), and the product is uncontaminated with any compounds of phosphorus.[5]

4. At the 0.2-mole level the yield is 82% (b.p. 159°) based on acetic acid.[5] The yield of ethyl bromoacetate-2-C^{14} is 4.0 g. (23.95 mmoles) based on 16.5 mmoles of barium carbonate-C^{14} (and 9.79 mmoles of acetic anhydride).

5. The procedure is a combination of the methods of Lapworth[6] (85%) and of Ward[7] (80%).

6. Omission of the hydrogen chloride and acetyl chloride lowers the yield to 40–63%.

7. The yield was determined by converting the product, without isolating, to the derivative 2,4-dichlorophenoxyacetic-1-C^{14} acid (q.v.). Pure bromoacetic acid[6] boils at 108–110° (30 mm.).

8. Evans[8] has found a slow rate of exchange between acetic acid and acetic anhydride. The half-time is about 10 hours for acetic anhydride in solvent acetic acid, or about 5 hours for acetic acid in acetic anhydride.

Bromoacetic-2-C^{14} acid has been prepared by Radin[9] by a modification of this method involving the bromination of bis(acetic-2-C^{14}) anhydride itself.

9. The procedure is adapted from the method of Ward.[7]

10. The purity is determined by isotope dilution.[10] An aliquot is diluted with a known amount of the pure nonisotopic compound, then converted to a suitable derivative which is purified to constant specific activity and assayed. Benzylamine acetate-2-C^{14}, m.p. 97–97.5°, was prepared[11] and crystallized from acetone.

11. The procedure is adapted from the method of Aschan.[12]

12. The boiling point of ethyl bromoacetate[1] is 154–155° (759 mm.).

C. Other Preparations

By procedures similar to Method V are prepared[13] bromoacetyl-1-C^{13} chloride (80%) and bromoacetic-1-C^{13} acid (80%). Acetyl-2-C^{14} chloride is brominated and converted to ethyl bromoacetate-2-C^{14} (see Method V); yield[14] 42% based on sodium acetate-2-C^{14}. Methyl bromoacetate-2-C^{14} has been prepared in 97% yield by esterification of bromoacetic-2-C^{14} acid with diazomethane. A diagram of the apparatus and detailed directions are given by Saunders.[15]

The preparation of sodium bromoacetate-2-C^{14} by a combination of Methods III and IV has been described by Freudenberg.[16] To a mixture of 0.25 ml. of bromine, 0.080 g. of acetic anhydride, 0.0005 g. of red phosphorus and a trace of pyridine is added by vacuum transfer the acetic-2-C^{14} acid liberated from 0.025 g. of sodium salt by dry hydrogen chloride. The mixture is heated at 125° for 2 hours in a sealed tube; then the bromine and hydrogen bromide are removed in a stream of carbon dioxide, and the residue is treated with 0.080 g. of sodium hydroxide in a little water.

[1] *Organic Syntheses*, Vol. 23, Wiley, New York, 1943, p. 37; S. Natelson and S. Gottfried, J. Am. Chem. Soc., *61*, 970 (1939).

[2] K. Auwers and R. Bernhardi, Ber., *24*, 2209 (1891); L. Vanino, *Handbuch der präparativen Chemie*, Vol. II, Enke, Stuttgart, 1937, p. 108.

[3] R. W. Holley, F. P. Boyle and D. B. Hand, Arch. Biochem. Biophys., *27*, 143 (1950).

[4] R. Gianetto and L. Bouthillier, Can. J. Biochem. and Physiol., *32*, 154 (1954).

[5] B. D. Shaw, J. Chem. Soc., *123*, 2233 (1923).

[6] A. Lapworth, J. Chem. Soc., *85*, 41 (1904).

[7] C. F. Ward, J. Chem. Soc., *121*, 1161 (1922).

[8] E. A. Evans, J. L. Huston and T. H. Norris, J. Am. Chem. Soc., *74*, 4985 (1952).

[9]N. S. Radin, D. Rittenberg and D. Shemin, J. Biol. Chem., *184*, 745 (1950).
[10]D. Rittenberg and G. L. Foster, J. Biol. Chem., *133*, 737 (1940).
[11]C. A. Buehler, L. Carson and R. Edds, J. Am. Chem. Soc., *57*, 2181 (1935).
[12]O. Aschan, Ber., *46*, 2162 (1913); *45*, 1913 (1912).
[13]N. S. Olsen, A. Hemingway and A. O. Nier, J. Biol. Chem., *148*, 611 (1943).
[14]A. S. San Pietro, J. Biol. Chem., *198*, 639 (1952).
[15]B. C. Saunders and T. S. Worthy, J. Chem. Soc., *1953*, 1929; Nature, *169*, 38 (1952).
[16]K. Freudenberg and W. Fuchs, Ber., *87*, 1824 (1954).

CHLOROACETIC-1-C^{14} ACID

$$CH_3C^*OONa \xrightarrow[2.\ Ac_2O]{1.\ HCl} CH_3C^*OOH \xrightarrow[P,\ I_2,\ PCl_5]{Cl_2} CH_2ClC^*OOH$$

R. Ostwald, J. Biol. Chem., *173*, 207 (1948).

A. Procedure (Note 1)

A platinum boat containing 1.408 g. of anhydrous sodium acetate-1-C^{14} is placed in a horizontal glass tube and dried for 24 hours at 30 μ. The tube then is connected to three traps cooled with a Dry Ice-2-propanol mixture and protected with a calcium chloride drying tube. Anhydrous hydrogen chloride (Note 2) is passed slowly through the system, while the tube is heated gradually. The liberated acetic-1-C^{14} acid is vacuum-transferred from the traps to a small chlorination flask (Note 3) cooled with liquid nitrogen.

To the acetic-1-C^{14} acid is added 0.65 g. of acetic anhydride at room temperature, and the mixture is dried by refluxing for 30 minutes (Note 4). A mixture of 0.02 g. of iodine, 0.04 g. of red phosphorus and 0.08 g. of phosphorus pentachloride is added (Note 5); then dry chlorine is passed slowly through the system for 2.5 hours under reflux at 100° (Note 6). All volatile material from the condenser walls and gas inlet is collected back in the flask by vacuum distillation with liquid nitrogen-cooling and warming of the glass surfaces. Purification is effected by fractional vacuum-sublimation onto a cold finger condenser filled with powdered Dry Ice; yield 1.52 g. (67%), m.p. 60° (Note 7). The radiochemical yield is 67.7%, and 15% of the initial activity is recovered from the washings and residues. The molar specific activity is 72% of that of the starting material.

B. Notes

1. The procedure is an adaptation of the method of Brueckner[1]. Sunlight or ultraviolet irradiation is unnecessary with the catalyst employed.

2. The hydrogen chloride is dried by passage through concentrated sulfuric acid, Drierite, and anhydrous aluminum chloride (or phosphorus pentoxide).

3. A diagram of the chlorination flask is presented by Ostwald. A small tube, fitted with a gas inlet discharging near the bottom, is equipped with a sidearm surmounted by a water-cooled reflux condenser and a low temperature cold finger cooled with Dry Ice-2-propanol and protected by a calcium chloride guard tube. See leucine-2-C^{14}, Figure II, 9.

4. Acetyl chloride (0.2 ml.) has been used as the dehydrating agent by many investigators.[2,3,4,5] The acetic-1-C^{14} acid contains 10–15% water and considerable hydrogen chloride.

5. The phosphorus pentachloride is omitted by some investigators.[3,4]

6. Tank chlorine is dried over phosphorus pentoxide and passed through a bubbler at a rate of about 5 ml. per minute.

7. The yield in trial runs was 85–90%. Ethyl chloroacetate-1-C^{14} has been prepared by Dauben[4] in 75–90% yield based on acetate-1-C^{14}. The crude acid is extracted from aqueous solution with ether and esterified with an ethereal solution of diazoethane. The excess reagent is distilled, and the ethereal solution is dried over potassium carbonate and distilled, b.p. 144°.

C. Other Preparations

Bromoacetic-1-C^{14} acid is prepared[6] in a similar manner at the 4.8-mmole level, employing 0.1 ml. of acetyl chloride, 0.020 g. of red phosphorus, a crystal of iodine and 0.5 ml. of bromine.

2-Bromopropionic-1-C^{14} acid is prepared,[7] in a similar manner, from sodium propionate-1-C^{14}, propionyl chloride (dehydrating agent), red phosphorus, iodine and bromine.

The preparation of chloroacetic-1-C^{14} or -2-C^{14} acid, by essentially this method, has been described by Dauben,[2] Hughes,[3,7] Chin,[8] Schayer (yield 56%, m.p. 58° from ligroin),[9] Noller[4] and Tolbert.[5]

Chloroacetic-1-C^{14} acid has been prepared in 60–90% yield by Mahler,[10] by chlorinating in nitrobenzene by a modification of the procedure described.

The preparation of ethyl chloroacetate-2-C^{14}, in 70–80% yield based on sodium acetate-2-C^{14} at the 22.5-mmole level, has been described by Noller.[6] The chloroacetic-2-C^{14} acid, prepared in the described manner, was converted to the potassium salt, dried and treated with ethyl sulfate. The mixture was refluxed for 2 hours at 135°, then distilled during 2 hours with a bath at 170°, employing decane as a chaser.

[1]H. Brueckner, Z. angew. Chem., *40*, 973 (1927); *41*, 226 (1928).
[2]W. G. Dauben and M. Gee, J. Am. Chem. Soc., *74*, 1078 (1952).

[3]D. M. Hughes and B. M. Tolbert, Atomic Energy Commission Report, AECU-121; Nuc. Sci., Abstracts, *2*, 1344 (1949).
[4]R. M. Noller and B. M. Tolbert, Atomic Energy Commission Report, UCRL-2041; Nuc. Sci. Abstracts, *7*, 2533 (1953).
[5]B. M. Tolbert and D. M. Hughes, Atomic Energy Commission Report, UCRL-705; Nuc. Sci. Abstracts, *4*, 5207 (1950).
[6]A. E. Almond, A. M. Burditt, D. E. Douglas and J. Eccles, Can. J. Chem., *33*, 679 (1955).
[7]D. M. Hughes, R. Ostwald and B. M. Tolbert, J. Am. Chem. Soc., *74*, 2434 (1952).
[8]D. Chin and P. Adams, Atomic Energy Commission Report, UCRL-2394; Nuc. Sci. Abstracts, *8*, 1057 (1954).
[9]R. W. Schayer, J. Am. Chem. Soc., *74*, 2441 (1952).
[10]H. R. Mahler, R. J. Speer and A. Roberts, Science, *110*, 562 (1949).

SODIUM FLUOROACETATE-2-C^{14}

$$BrC^*H_2COOCH_3 \xrightarrow{KF} \underset{(a)}{FC^*H_2COOCH_3} \xrightarrow{NaOH} \underset{(b)}{FC^*H_2COONa}$$

B. C. Saunders and T. S. Worthy, Nature, *169*, 38 (1952); J. Chem. Soc., *1953*, 1929.

A. Procedure (Note 1)

(a) *Methyl Fluoroacetate-2-C^{14}.* Into a stainless steel, electrically-heated microautoclave (Figure II, 12) containing 0.065 g. of potassium fluoride (Note 2) and 12–14 stainless steel balls (3/16"), is vacuum-distilled 0.110 g. of methyl bromoacetate-2-C^{14} with liquid nitrogen cooling. The autoclave is brought to atmospheric pressure with dry air, detached from the vacuum system, sealed and mechanically shaken in a vertical plane for 3.5 hours at 220°. The mixture is vacuum-transferred into a small tube; the volatile product contains only unreacted methyl bromoacetate-2-C^{14} and 47–72% methyl fluoroacetate-2-C^{14} (Note 3). The mixture is fractionated by vacuum distillation at –25° in a continuously evacuated system, the methyl bromoacetate-2-C^{14} being condensed in a trap at –54°, while the more volatile product is collected in a second trap cooled with liquid nitrogen (Note 4). The yield is 40–60%, and the purity is 94–100% (Note 5).

(b) *Sodium Fluoroacetate-2-C^{14}.* To 0.042 g. of methyl fluoroacetate-2-C^{14}, transferred to a titration cell under high vacuum (Note 2), is added 1 ml. of water. The cell is equipped with a pressure-equalizing burette, a glass electrode and a tube connected to a calomel half-cell. The magnetically stirred mixture is titrated slowly to pH 11 with *N* sodium hydroxide (0.43 ml., calc. 0.45) (Note 6).

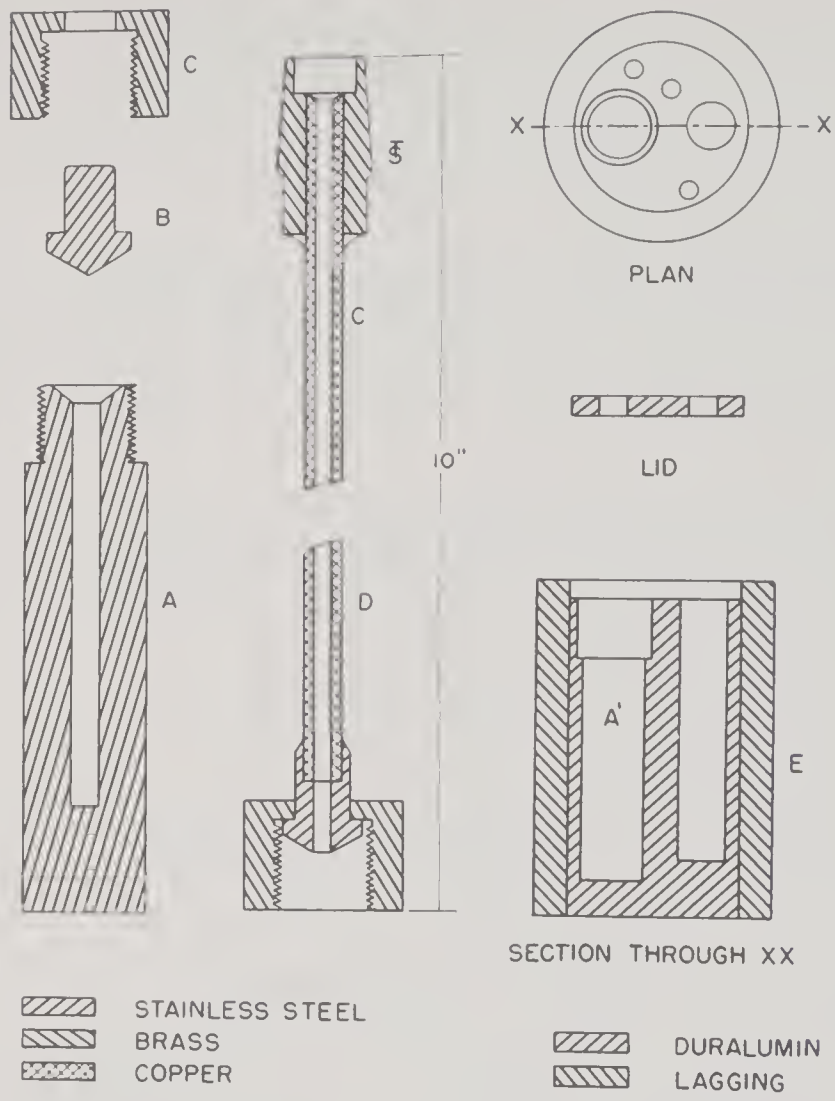


Fig. II, 12. Microautoclave (B. C. Saunders and T. S. Worthy). A, Body with 120° socket and containing 12 stainless steel balls (diam. 3/16 in.); B, cap (with slightly more acute taper); C, locking nut; D, filling attachment fitted with copper tube and ⅛ brass adapter to vacuum system; E, lagged heating block with holes for autoclave and 50-W heater. The entire assembly is shaken mechanically in a vertical plane.

The resulting solution is removed and evaporated to dryness by freeze-drying; yield 0.045 g.

B. Notes

1. The procedure is an adaptation of the method described by Saunders and Stacey.[1] Bromoacetic acid cannot be fluorinated directly, and fluorination of the ester at ordinary pressure gives poor yields.

2. The system is pumped to high vacuum for 2 hours to remove any water.

3. There is some decomposition of methyl bromoacetate at this temperature, for only 89% of the ester is recovered unchanged in a run without the fluoride. The composition is estimated by comparing the values of the refractive index with those of known mixtures, the accuracy being confirmed by fluorine analysis; methyl fluoroacetate n_D^{15} 1.3700, methyl bromoacetate n_D^{15} 1.4582.

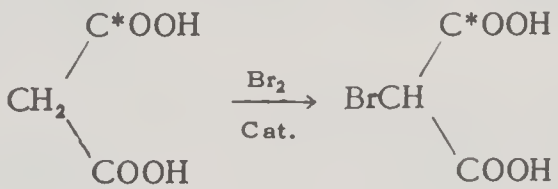
4. The cooling baths are melting carbon tetrachloride (–25°) and melting ethyl malonate (–54°). Preliminary experiments with known mixtures, containing 47–49% of methyl fluoroacetate, have shown that this method of micro-fractionation gives a sample of 94–100% purity in 77–57% recovery.

5. The infrared spectrum of the vapor corresponds with that of an artificial mixture containing 95–99% of methyl fluoroacetate.

6. As the equivalent weights of methyl bromoacetate and methyl fluoroacetate are sufficiently different, the value of the titre is a measure of the purity of the product.

[1]B. C. Saunders and G. J. Stacey, J. Chem. Soc., *1948*, 1773.

BROMOMALONIC-1-C^{14} ACID



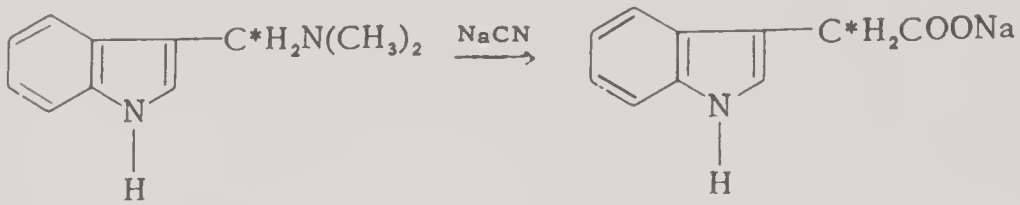
P. E. Yankwich and E. C. Stivers, J. Chem. Phys., *21*, 61 (1953).

Procedure

To a stirred mixture of 10.0 g. of malonic-1-C^{14} acid and 75 ml. of moist ether is added 0.001 g. of benzoyl peroxide, followed dropwise by 18.7 g. (6 ml.) of bromine. The greenish amber solution is evaporated to dryness under vacuum (72 hours). The crude product is dissolved in 6 ml. of anhydrous trifluoroacetic acid at 85°, care being taken to exclude moisture in all subsequent operations. The filtered solution is evaporated to dryness under vacuum, and the residue is redissolved in 6 ml. of the solvent. The solution is filtered, then frozen with a Dry Ice-alcohol bath and warmed to room temperature to accomplish the fractionation of product by utilizing the slowness with which pure bromomalonic acid dissolves in the solvent at room temperature. The product is recrystallized three times, and the last trace of solvent is evaporated under reduced pressure at room temperature, 72 hours; yield 47%, m.p. 112–113° (dec.). The melting point is raised to 116–117° by heating the acid under vacuum for 3 hours at 90°.

3-INDOLEACETIC-α-C^{14} ACID
(C_1^{14}-Heteroauxin)

METHOD I

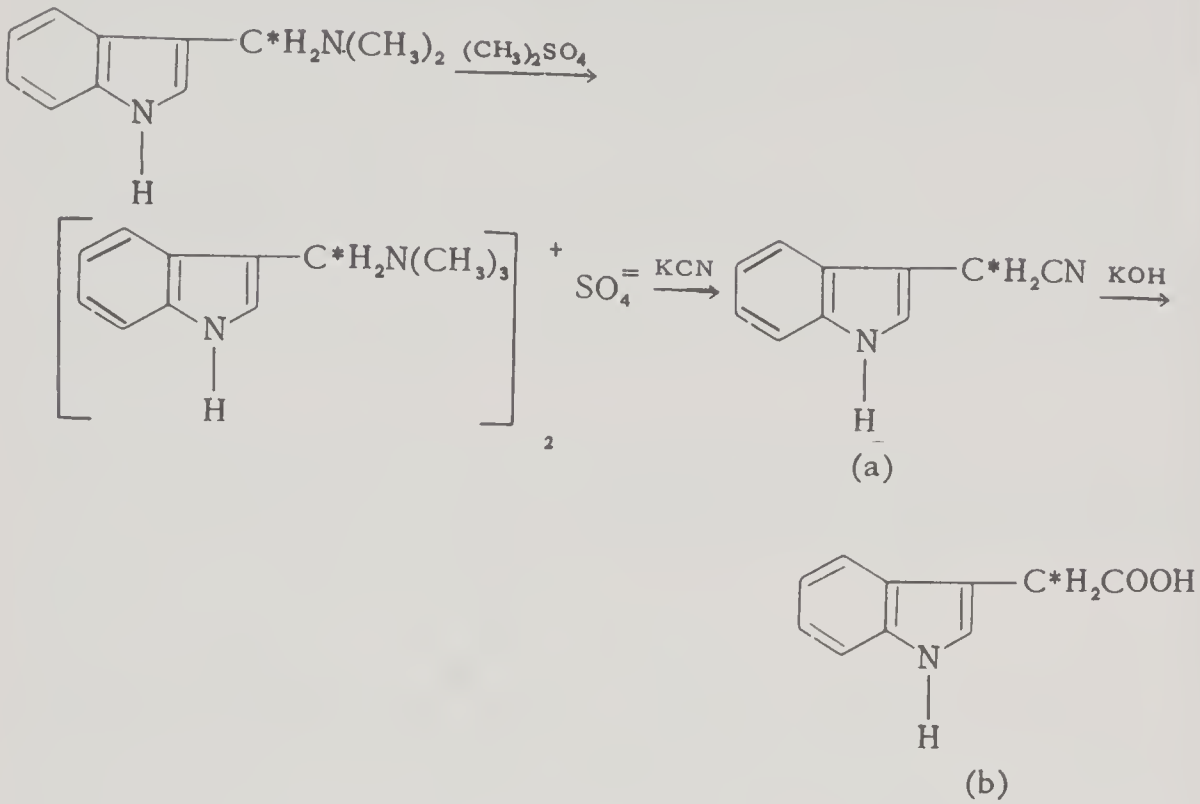


R. E. Stutz, D. E. Atkinson and S. A. Gordon, Atomic Energy Commission Report, ANL–4710; Nuclear Sci. Abstr. *6*, 164 (1952).

A. Procedure (Note 1)

An aqueous solution containing 3.5 mmoles of 3-(dimethylaminomethyl-C^{14})indole hydrochloride is cooled in an ice-bath, then adjusted to pH 10 and extracted with four portions of ether. The extract is evaporated with an air-stream inside a 25 × 100-mm. Pyrex bomb. To the residual C_1^{14}-gramine is added 1.96 g. (40 mmoles) of sodium cyanide and 10 ml. of 50% methanol; then the tube is cooled in liquid nitrogen, evacuated for 5 minutes to remove condensed oxygen, and sealed. The mixture is autoclaved at 125° (Note 2) for 16–18 hours, then transferred into a 100-ml. flask by washing with water and acetone. The methanol, acetone and water are removed by lyophilizing (9 hours), and the residue is dissolved in water. The solution is washed with two portions of ether, then adjusted to pH 3 and extracted with four portions of ether. The ether is evaporated over 5 ml. of 2 *N* sodium hydroxide, and the aqueous solution is adjusted to pH 3 and cooled. The product is collected on a filter, washed with cold water and dried over phosphorus pentoxide; yield 0.572 g., m.p. 164–166°, 85–90% (Note 3). The presence of 2–3% of impurities (Note 4) is indicated by a colorimetric determination[1] of indoleacetic acid, a comparison of the ultraviolet absorption spectra and by paper chromatography and radioautography (Note 5).

METHOD II



C. Heidelberger, J. Biol. Chem., *179*, 139 (1949).

A. Procedure (Note 6)

(a) *3-Indoleacetonitrile-α-C*^{14}. To a solution of 0.050 g. of 3-(dimethylaminomethyl-C^{14})indole and 0.038 g. of potassium cyanide in 1 ml. of 50% ethanol is added 0.1 ml. of methyl sulfate, and the mixture is refluxed for 1.5 hours. The solvent is distilled, the residual yellowish oil is extracted into ether solution, and the ether is evaporated.

(b) *3-Indoleacetic-α-C*^{14} *Acid.* The crude 3-indoleacetonitrile-α-C^{14} is refluxed for 48 hours with 0.050 g. of potassium hydroxide in a solution of 2.5 ml. of ethanol and 0.5 ml. of water. The alcohol is evaporated, water is added, and the alkaline solution is washed with ether and acidified. The insoluble product is collected and recrystallized from water; yield 0.025 g. (50%), m.p. 163–165° (Note 7).

B. Notes

1. The procedure is a modification of the method of Snyder and Pilgrim,[2] eliminating the 80-hour reflux period and the subsequent hydrolysis of the by-product, 3-indoleacetamide, or the nitrile (Method II).

2. The autoclave pressure is 20 p.s.i.

3. The yield is 69–71% based on barium carbonate at the 4.6-mmole scale (the gramine being prepared in 75–80% yield by use of vacuum-line techniques, without mechanical transfer). When purified gramine was used in trial runs, the product melted consistently at 168–170°.

4. Partition between water and ether at high and low pH, followed by recrystallization from ether-ligroin, does not significantly alter the melting point or specific activity. Purification may be accomplished effectively by sublimation at 140–150° (1 μ).[3]

5. The paper chromatogram is developed with 1-butanol, saturated with dilute aqueous hydrochloric acid or ammonium hydroxide. Two secondary spots were markedly reduced or missing, when the chromatogram was developed under nitrogen.

6. The procedure is a modification of the method of Snyder.[4] The quarternary ammonium salt, formed by the reaction of gramine and methyl sulfate, is a more reactive alkylating agent than those containing the simple benzyl group.

7. The acid is stored best as the sodium salt. The over-all yield of 16% based on carbon dioxide was confirmed by Stutz, Atkinson and Gordon.

C. Other Preparations

The preparation of 3-indoleacetic-α-C^{14} acid by a variation of Method I has been described by Weygand[5,2] at the 0.7-mmole scale; yield 75.4%, m.p. 167–168°. C_1^{14}-Gramine and sodium cyanide are refluxed with aqueous alcohol for 95 hours, and the residual 3-indoleacetamide-α-C^{14}, obtained by evaporation, is refluxed for 5 hours with aqueous sodium hydroxide. Acidification gives the product which is crystallized from dichloroethylene.

The preparation of 3-indoleacetic-2-C^{14} acid, also by the above variation of Method I, has been described by Pichat;[3] yield 77%, m.p. 167–169°. The sublimed colorless product developed a pink hue after standing for several weeks at room temperature in the dark in a sealed tube; resublimation again gave a colorless product.

[1]S. A. Gordon and R. P. Weber, Plant Physiol., *26*, 192 (1951); Chem. Abstracts, *45*, 4605 (1951).

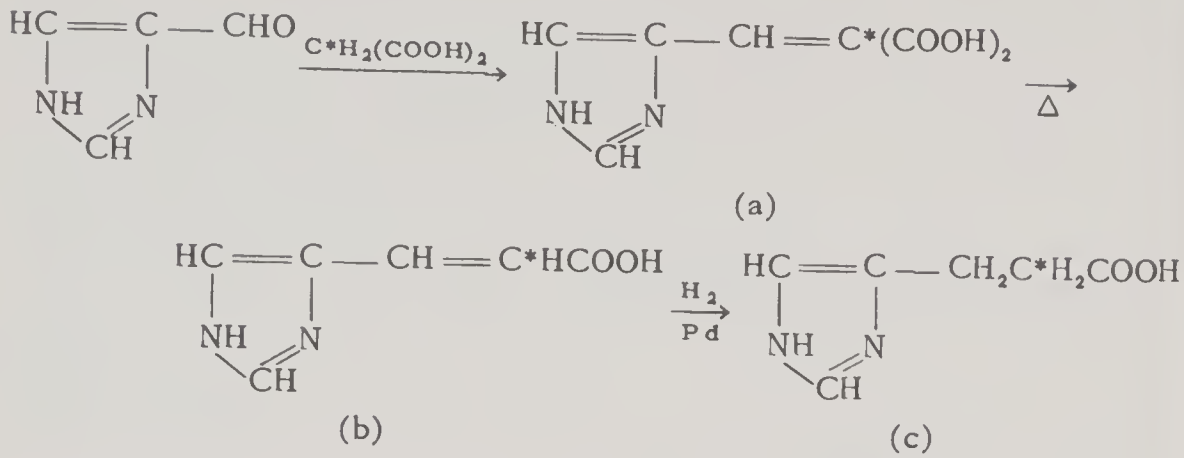
[2]H. R. Snyder and F. J. Pilgrim, J. Am. Chem. Soc., *70*, 3770 (1948).

[3]L. Pichat, M. Audinot and J. Monnet, Bull. soc. chim. France, (5) *21*, 85 (1954).

[4]H. R. Snyder, C. W. Smith and J. M. Stewart, J. Am. Chem. Soc., *66*, 200 (1944).

[5]F. Weygand and H. Lerner, Z. Naturforsch., *9b*, 682 (1954).

4(or 5)-IMIDAZOLEPROPIONIC-α-C^{14} ACID



M. Kraml and L. P. Bouthillier, Can. J. Biochem. and Physiol., 33, 590 (1955).

A. Procedure

(a) *4(or 5)-Imidazolemethylenemalonic-α-C^{14} Acid* (Note 1). A mixture of 0.54 g. of 4(or 5)-imidazolecarboxaldehyde (Note 2), 5.6 mmoles of malonic-2-C^{14} acid and 10 ml. of water is heated at 50° for 4 hours. The product precipitates almost quantitatively, m.p. 206–208° (dec.).

(b) *Urocanic-α-C^{14} Acid*, [*4(or 5)-Imidazoleacrylic-α-C^{14} Acid*]. A suspension of the crude malonic acid in 100 ml. of pyridine is refluxed for 14 hours. The solution is evaporated under vacuum, and the residue is recrystallized (Norit) from 25 ml. of hot water as the dihydrate; m.p. 230–231° (Note 3). Purity is demonstrated by the formation of only one radioactive peak coincident with the Pauly diazo-positive spot during paper chromatography in four solvents (Note 4).

(c) *4(or 5)-Imidazolepropionic-α-C^{14} Acid.* Urocanic-α-C^{14} acid (0.085 g.) in 50 ml. of water is hydrogenated for 4 hours at one atmosphere over 0.100 g. of 5% palladium-on-calcium carbonate (Note 5). The absence of starting material and purity of the product are demonstrated by paper chromatography (see Note 4, c), R_f 0.45. The product is crystallized as the dihydrate from water-ethanol-acetone, m.p. 204–206°.

B. Notes

1. The procedure is an adaptation and modification of that of Akabori.[1]

2. For preparation of the starting material see[1] α-amino-4(or 5)-imidazolepropionic-C^{14} acid, Note 3.

3. The ultraviolet absorption spectrum in 0.1 *N* hydrochloric acid (pH 1.0), 0.01 *M* phosphate buffer (pH 7.4) and distilled water (pH 4.6) agrees with that described by Mehler.[2] The yield is 64% at the 7.5-g. scale.[1]

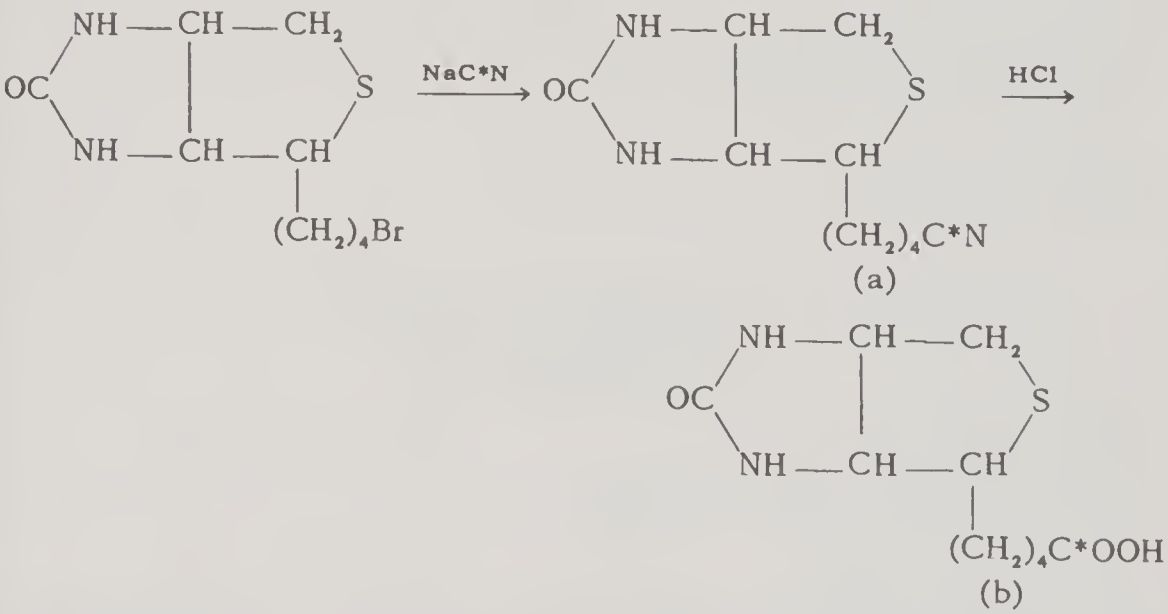
4. One-dimensional paper chromatograms are developed on Whatman paper No. 1 by the ascending method[3] employing: a) 1-butanol-glacial

acetic acid-water (15:3:7);[4] b) 1-butanol-95% ethanol-concentrated ammonium hydroxide (8:1:3);[5] c) benzene-1-butanol-methanol-water (1:1:2:1) (R_f 0.65); and d) phenol-water (4:1, in the presence of ammonia and sodium cyanide) or saturated with borate buffer (pH 9.3).[7] The presence of imidazole compounds is detected by spraying with freshly diazotized 0.5% sulfanilic acid and dusting with sodium carbonate, a red spot being formed.

5. Akabori[1] carried out the reduction of urocanic acid with 2.3% sodium amalgam in the presence of an excess of aqueous acetic acid. The hydrochloride melts at 83°.

[1]S. Akabori, S. Ose and T. Kaneko, Proc. Imp. Acad. (Tokyo), *16*, 191 (1950).
[2]A. H. Mehler and H. Tabor, J. Biol. Chem., *201*, 775 (1953).
[3]R. J. Williams and H. Kirby, Science, *107*, 481 (1948).
[4]J. L. Auclair and J. B. Maltais, Nature, *170*, 1114 (1952).
[5]R. W. Schayer, J. Biol. Chem., *196*, 469 (1952).
[6]M. Mason and C. P. Berg, J. Biol. Chem., *188*, 783 (1951).
[7]A. L. Levy and D. Chung, Anal. Chem., *25*, 396 (1953).

D-*cis*-TETRAHYDRO-2-OXOTHIENO[3,4]IMIDAZOLINE-4-VALERIC-C^{14} ACID (C_1^{14}-D-Biotin)



S. D. Baker, D. E. Douglas and A. E. Seath, Atomic Energy Commission Report, NP-3178; Nuclear Sci. Abstr., *5*, 5158 (1951).

A. Procedure (Note 1)

(a) *D*-cis-*Tetrahydro-2-oxothieno*[*3,4*]*imidazoline-4-valeronitrile-*C^{14}. A mixture of 3.7 mmoles of sodium cyanide-C^{14}, 1 equivalent of excess sodium hydroxide, 2 ml. of water, 5.2 ml. of alcohol and 0.58 g. of D-*cis*-

4-(4-bromobutyl)tetrahydro-2-oxothieno[3,4]imidazoline (Note 2) is refluxed for 20 hours. The mixture is evaporated to dryness at 50° under reduced pressure (Note 3).

(b) *D-cis-Tetrahydro-2-oxothieno[3,4]imidazoline-4-valeric-C*^{14} *Acid.* The crude nitrile is refluxed for 2 hours with 30 ml. of 6 *N* hydrochloric acid, liberated hydrogen cyanide-C^{14} being trapped in alkali. The mixture is evaporated at 50° under reduced pressure, and the residue is extracted with dilute sodium hydroxide solution (Note 4). The aqueous solution is treated with carbon and acidified to precipitate the crude product. Purification is achieved by reprecipitation and recrystallization from boiling water; yield 0.0428 g. (Note 5). A further 0.1-g. crop of impure product may be recovered from the mother liquors.

B. Notes

1. The procedure is essentially that of Grüssner.[1]

2. Directions are given by Grüssner[1] for preparing the starting material.

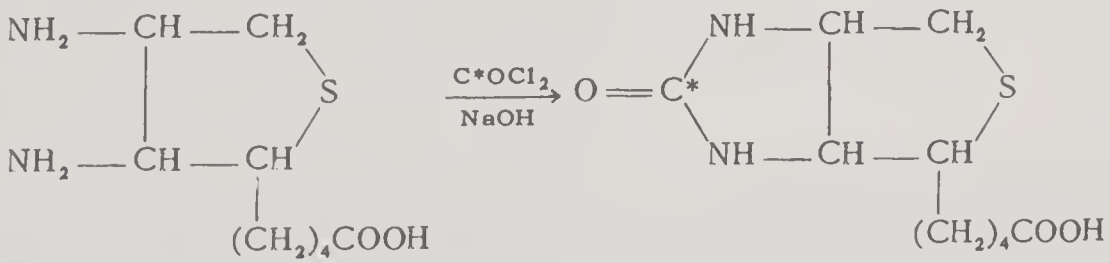
3. The nitrile, after being washed with water, may be recrystallized from methanol-water or methanol-isopropyl alcohol, m.p. 210–212° (cor.).

4. Considerable neutral gummy residue is left behind, the amount being related to the amount of alkali present during the nitrile formation.

5. The best yield is 22%, but over-all yields of 60% were obtained in trial runs in the absence of added alkali. There is no depression upon mixed melting with an authentic sample. Biotin melts at 221–222° (cor.);[1] the water solubility is 22 mg. per 100 ml. at 25°.

[1]A. Grüssner, J. P. Bourquin and O. Schnider, Helv. Chim. Acta, *28*, 517 (1945).

D-*cis*-TETRAHYDRO-2-OXOTHIENO[3,4]IMIDAZOLINE-4-VALERIC-2-C^{14} ACID (C_1^{14}-D-Biotin)



D. B. Melville, J. G. Pierce and C. W. H. Partridge, J. Biol. Chem., *180*, 299 (1949).

A. Procedure (Note 1)

The phosgene-C^{14} prepared from 0.1012 g. of barium carbonate-C^{14} (Note 2) is transported with a stream of nitrogen and condensed, by means

of a liquid nitrogen-bath, into a flask containing a frozen mixture of 1.5 ml. of 1.7 *N* sodium hydroxide and 0.050 g. of the diaminocarboxylic acid sulfate derived from D-biotin (Note 3). The flask is evacuated briefly and closed off by means of a stopcock; then the mixture is warmed to room temperature and shaken for 1 hour. The solution is acidified (Note 4), and the precipitated product is collected and recrystallized from water; yield 0.0104 g., m.p. 226–230° (Note 5). An additional 0.025 g. is obtained from the mother liquor with the aid of carrier. The radiochemical yield is 33% based on unrecovered carbon-C^{14} dioxide (Note 6).

B. Notes

1. The procedure is according to the method of Melville.[1]

2. The phosgene is prepared from carbon-C^{14} monoxide obtained by the dehydration of formic-C^{14} acid, which is synthesized by the reduction of bicarbonate-C^{14}. The compound is swept with nitrogen through a tube of mercuric sulfide to remove excess chlorine.

3. The preparation of the starting material, by hydrolysis of D-biotin, is described by Hofmann.[2]

4. The liberated carbon dioxide, equivalent to 56% of the starting barium carbonate-C^{14}, is collected in a trap containing alkali.

5. The yield in trial runs, based on the diaminocarboxylic acid, was 98%, m.p. 228–230°.

6. No attempt has been made to improve the yield in the last step of the synthesis.

[1]D. B. Melville, K. Hofmann and V. du Vigneaud, Science, *94*, 308 (1941).

[2]K. Hofmann, D. B. Melville and V. du Vigneaud, J. Biol. Chem., *141*, 207 (1941).

SODIUM PYRUVATE-1-C^{13}

$$CH_3CHO \xrightarrow{NaC^*N} CH_3CHOHC^*N \xrightarrow[(NaOH)]{HCl} CH_3CHOHC^*OONa \xrightarrow[H_2SO_4]{n\text{-}C_4H_9OH} CH_3CHOHC^*OOC_4H_9$$

(a) (b)

$$CH_3CHOHC^*OOC_4H_9 \xrightarrow{KMnO_4} CH_3COC^*OOC_4H_9 \xrightarrow{NaOH} CH_3COC^*OONa$$

(c) (d)

W. Sakami, W. E. Evans and S. Gurin, J. Am. Chem. Soc., *69*, 1110 (1947).

A. Procedure

(a) *Sodium Lactate-1-*C^{13} (Note 1). To an ice-cold solution of 1.00 g. of sodium cyanide-C^{13} in 4 ml. of water is added 10.3 ml. of ice-cold 2.15

N sulfuric acid, followed by 1.4 ml. of acetaldehyde and 2.0 ml. of 1.00 *N* sodium hydroxide. The well-shaken mixture is set aside at room temperature for 20 minutes, then treated with 40 ml. of concentrated hydrochloric acid, and the lactonitrile-1-C^{13} is hydrolyzed by refluxing for 6 minutes. The mixture is diluted with 100 ml. of water, cooled, basified with saturated sodium hydroxide solution and distilled to remove ammonia. The residual solution is neutralized with sulfuric acid and concentrated until bumping commences. Toluene (50 ml.) is added, and the residual water is removed azeotropically (Note 2). The mixture is evaporated to dryness, the residue is extracted with three 50-ml. portions of absolute alcohol, and the solvent is distilled. The residue is extracted into 15 ml. of absolute alcohol, and the solid is separated by centrifuging and washed with three 1-ml. portions of the alcohol. The combined solution is concentrated by distillation, and the residue is dried in a vacuum desiccator; yield 94–96% (Note 3).

(b) *Butyl Lactate-1-C^{13}*. A mixture of the crude sodium lactate-1-C^{13} and 11 ml. of 2.5 *N* sulfuric acid in butyl alcohol is refluxed for 12 hours with benzene, with use of a trap to collect the water formed. The benzene is distilled, the sulfuric acid is neutralized with sodium butoxide in butyl alcohol (Note 4), and the sodium sulfate is removed by centrifuging and washed with 1-butanol. The solutions and washings are combined and vacuum-distilled to remove a small amount of nonvolatile gum. The product is collected by fractional redistillation of the distillate at atmospheric pressure (Note 5); yield 80% based on sodium lactate.

(c) *Butyl Pyruvate-1-C^{13}* (Note 6). To an ice-cold solution of 26.14 ml. of 0.167 *M* potassium permanganate and 4.44 ml. of 1.96 *N* sulfuric acid is added 1.94 g. of butyl lactate-1-C^{13}, followed by 30 ml. of cold water with shaking. The mixture is stored at 18–20° for 3 hours, then chilled, basified with 5 ml. of cold sodium carbonate solution and exhaustively extracted with ether. The extract is dried over calcium chloride and concentrated, and the residue is distilled at a bath temperature of 190–205°; yield 1.41 g. (74%), purity 95% (Note 7).

(d) *Sodium Pyruvate-1-C^{13}*. The ester is titrated at room temperature with *N* sodium hydroxide (Note 8). The 1-butanol is extracted with ether, and the aqueous solution is freed from ether by aeration (Note 8); yield 50–55% based on cyanide.

B. Notes

1. The procedure is a modification of the method of Cramer and Kistiakowsky.[1] For another preparative method see lactic-2-C^{14} acid, Other Preparations.

2. The azeotrope is distilled continuously with use of a water trap, such as is illustrated by Natelson.[2]

3. The lactic acid is determined by oxidation to acetaldehyde and titration of bound bisulfite, as described by Friedmann and Graeser.[3]

4. The blue color of benzeneazo-α-naphthylamine (0.1% in 1-butanol) is a satisfactory end point for this titration.

5. The small fractionating column is equipped with a jacket maintained at 120°. Butyl lactate boils at 70.5–73° (10–11 mm.).

6. The procedure is adapted from a German patent.[4]

7. Butyl pyruvate boils at 75–76° (12 mm.); the phenylhydrazone melts at 64°. The pyruvate is determined by titration with cerium by the procedure of Fromageot.[5] A colorimetric determination is described by Friedmann and Haugen,[6] and a determination by decarboxylation with yeast has been reported by Strecker.[7]

8. The saponification of the pyruvic ester is rapid. Due to the instability of pyruvic acid, the solution should be used as soon as possible (see pyruvic-2-C^{14} acid, Note 5).

C. Other Preparations

In a similar manner, starting with acetaldehyde-C_2^{13}, are prepared: sodium lactate-2,3-C_2^{13}, butyl lactate-2,3-C_2^{13}, butyl pyruvate-2,3-C_2^{13} and sodium pyruvate-2,3-C_2^{13}.

The preparation of lactic-1-C^{11} acid, by a modification of this method, has been described by Cramer[1] (yield 40%) and Nahinsky.[8]

Lactic-1-C^{14} acid has been prepared in 81.8% yield by Hughes, Ostwald and Tolbert; see zinc lactate-2-C^{14}.

The preparation of lactic-2,3-C_2^{11} acid from acetaldehyde-C_2^{11}, by a modification of the method described, has been reported by Cramer[1] (50% yield) and Veenesland[9] (20% yield).

Pyruvic-1-C^{14} acid has been prepared by Wood[10] in 20–40% over-all yield based on sodium cyanide-C^{14}. Cuprous cyanide-C^{14} was reacted with acetyl bromide, and the pyruvonitrile-1-C^{14} was hydrolyzed to pyruvamide-1-C^{14} and purified by sublimation, prior to hydrolysis to the acid (see pyruvic-2-C^{14} acid). Christensen[11] found that hydrolysis of pyruvonitrile-1-C^{14} with a stoichiometric amount of water in ethereal hydrogen chloride gave pyruvamide-1-C^{14} in reproducible yields of 70%. Hydrolysis of the latter was straightforward, giving a 75% yield of purified pyruvic-1-C^{14} acid. A similar preparation has been reported by Foster[12] and Kobayashi.[13]

[1]R. D. Cramer and G. B. Kistiakowsky, J. Biol. Chem., *137*, 549 (1941).
[2]*Organic Syntheses*, Vol. 23, Wiley, New York, 1943, p. 38, Fig. 3.
[3]T. E. Friedmann and J. B. Graeser, J. Biol. Chem., *100*, 291, (1933).
[4]Byk-Gouldenwerke, Chem. Fabrik, A.- G., D.R.P. 526, 366; Chem. Abstracts *25*, 4285 (1931).

[5]C. Fromageot and P. Desnuelle, Biochem. Z., *279*, 174 (1935); H. A. Krebs and W. A. Johnson, Biochem. J., *31*, 645 (1937).
[6]T. E. Friedmann and G. E. Haugen, J. Biol. Chem., *147*, 415 (1943).
[7]H. J. Strecker, H. G. Wood and L. O. Krampitz, J. Biol. Chem., *182*, 525 (1950).
[8]P. Nahinsky, C. N. Rice, S. Ruben and M. D. Kamen, J. Am. Chem. Soc., *64*, 2299 (1942).
[9]B. Veenesland, A. K. Solomon, J. M. Buchanan, R. D. Cramer and A. B. Hastings, J. Biol. Chem., *142*, 371 (1942).
[10]H. G. Wood, Nucleonics, 7 (3), 60 (1950).
[11]B. E. Christensen, Nucleonics, 7, (3), 60 (1950).
[12]J. M. Foster and C. A. Villee, J. Biol. Chem., *211*, 797 (1954).
[13]Y. Kobayashi and G. Kalnitsky, J. Biol. Chem., *211*, 473 (1954).

PYRUVIC-2-C^{14} ACID

$$CH_3C^*OBr \xrightarrow{Cu_2(CN)_2} \underset{(a)}{CH_3C^*OCN} \xrightarrow{HCl} \underset{(b)}{CH_3C^*OCONH_2} \xrightarrow{HCl} \underset{(c)}{CH_3C^*OCOOH}$$

H. S. Anker, J. Biol. Chem., *176*, 1333 (1948).

A. Procedure (Note 1)

(a) *Pyruvonitrile-2-C^{14}.* Acetyl-1-C^{14} bromide (8 mmoles) is added to 9 mmoles of dry cuprous cyanide in an ice-cooled ampoule. Sufficient anhydrous cyclohexane is added to wet the powder, and the ampoule is sealed and stored for 3 days at room temperature. The mixture is extracted with five 2.5-ml. portions of absolute ether, and the extract is used directly (Note 2); yield 75-80%.

(b) *Pyruvamide-2-C^{14}* (Note 3). The ether solution of the nitrile is cooled with an ice-bath, then saturated with dry hydrogen chloride. Water (6.5 mmoles) is added, and hydrogen chloride is bubbled through the solution for 30 minutes. The crystalline product is collected by gravity filtration and washed with two 5-ml. portions of ether-cyclohexane (5:1) saturated with hydrogen chloride. The chloride is removed by connecting the funnel to a stream of dry nitrogen (3 hours), and the product is recrystallized from a minimal amount of dry ethyl acetate; yield 40–70%, m.p. 127° (Note 4).

(c) *Pyruvic-2-C^{14} Acid.* A solution of 1 mmole of pyruvamide-2-C^{14}, 1–3 ml. of water and 1 ml. of *N* hydrochloric acid is heated on a steam-bath for 1.5 hours (Note 5); yield 90–100% (Note 6).

B. Notes

1. The procedure is a modification of the method of Tschelinzeff and Schmidt[1] for preparing α-keto acids.

2. Pyruvonitrile boils at 93°, d_4^{20} 0.9754, n^{20} 1.3743.

3. The procedure is adapted from the method of Claisen and Shadwell.[2]

4. A yield of 79%, m.p. 123–124° (cor.), is reported by Thomas.[3] The acid hydrolysis of pyruvonitrile-2-C^{14} in aqueous media yields a mixture[3,18] of formic acid, isotopic acetic acid[4] (50%),[5] pyruvamide-2-C^{14} and pyruvic-2-C^{14} acid. Both pyruvamide-2-C^{14} and pyruvamide-1-C^{14}, prepared by this method, are often seriously contaminated with isotopic impurities.[6]

5. Due to instability of the acid, the solution should be used as soon as possible. Storage is best achieved in the form of the amide or the dry sodium salt. Paper chromatography[7] shows that after 3 months in a frozen solution, 78% of the radioactivity of the original sodium pyruvate-2-C^{14} appears in three new peaks.[4]

Chromatographic analysis of the product prepared in this manner indicates that there are present 5–10% of isotopic impurities.[8] Groth[6,9] and Busch[8] have found it desirable to purify the product by anion exchange chromatography to remove nonvolatile and neutral components. Directions are given by Groth[6] for the chromatographic procedure on Dowex-1 by elution with gradually increasing[10] concentrations of hydrochloric acid (50–85% of the activity is recovered in the pyruvic acid peak). Elution with formic acid solutions of gradually increasing acidity also is described by Busch.[10] The purified preparations contain less than 1% of labeled impurities, and are stable over a 6-month period, frozen in 1 *N* hydrochloric acid.

6. The pyruvic acid is determined colorimetrically; see sodium pyruvate-1-C^{13}, Note 7. Thomas[3] has reported a yield of 64% (65% radiochemical yield) with 98% purity. Zamecnik[4] has reported a radiochemical yield of 60%, although a larger fraction could be isolated as pyruvic acid dinitrophenylhydrazone. Purification[4] is achieved best through conversion to the sodium or potassium salt. The acid is extracted into ether, and the solution is dried over sodium sulfate, concentrated and fractionated (b.p. 60–75° at 25 mm.,[11] 60–63° at 10 mm.[12]). The salt then is precipitated from an absolute alcoholic solution of the acid by addition of a saturated ethanolic solution of sodium acetate. Alternatively, the acid may be titrate[11] in 10 volumes of methanol with methanolic potassium hydroxide, and the salt is precipitated by dilution with ether; the yield depends on the purity of the acid. Mosbach[13] has purified the sodium salt by partition chromatography; recovery 85%.

Ethyl pyruvate-2-C^{14} is prepared[14,15] by esterification in the presence of sulfuric acid catalyst; yield 65–80%, b.p. 50–55° (19 mm.). Thermal decomposition at 120° forms nonisotopic carbon monoxide and ethyl acetate-1-C^{14}.

C. Other Preparations

By the same procedures, starting with the appropriate acetyl bromide, are prepared the 3-C^{14}-labeled[16,17] and the 2,3-C_2^{13}- (or C^{14}-) labeled[18] pyruvonitrile, amide and acid.

Pyruvonitrile-2-C^{14} has been prepared by Calvin and Lemmon,[11,14,15] by a modification of the procedure described, yield 70–75% based on acetic-1-C^{14} acid at the 250-mmole level. The preparation by the procedure of Tschelinzeff and Schmidt[1] has been reported by Schepartz[19] and Gould[20] in 60% and 90% yield, respectively, based on acetyl bromide.

Pyruvamide-2-C^{14} has been prepared in 79% yield by Thomas,[3] by hydrolysis of the nitrile in an etheral medium.

The preparation of pyruvic-2-C^{14} acid by hydrolysis of the amide has been reported by Radin,[21] Strecker,[22] Thomas[3] (64% yield), Zamecnik[4] (60% yield), Schepartz[19] (40–70% yield based on nitrile), and by Wang[12] (42% based on acetate). Also see Arnstein.[23]

The preparation of pyruvic-2-C^{14} acid by hydrolysis of the nitrile has been investigated by Thomas,[3] Zamecnik,[4] Hughes and Reid,[5] Calvin and Lemmon[11,15] (25–52% yield), and by Gould.[20] The method appears to give rise to considerable isotopic acetic acid as a by-product.

The preparation of pyruvic-2-C^{14} acid from acetyl-1-C^{14} bromide by the described procedure has been reported by Foster[24] and Milhaud.[25] Paper chromatography (R_f 0.59 in phenol and R_f 0.43 in butanol-propionic acid) gave one major spot containing 98% of the total activity.[25] The acid has been converted[6] to hydroxypyruvic-2-C^{14} acid by bromination and hydrolysis of the bromopyruvic-2-C^{14} acid, according to the method of Sprinson.[26]

The preparation of pyruvic-3-C^{14} acid phenylhydrazone has been described by Leete.[17] To a solution of 0.834 g. (9.6 mmoles) of pyruvamide-3-C^{14} in 30 ml. of water is added a solution of 0.95 ml. (9.6 mmoles) of freshly distilled phenylhydrazine in 5 ml. of 2 *N* hydrochloric acid. The mixture is stored at 0° for 1 hour, and the pyruvamide-3-C^{14} phenylhydrazone (m.p. 143–144°) is collected on a filter washed with 100 ml. of water, then wetted with methanol, dissolved in 100 ml. of boiling water and diluted with 150 ml. of 2 *N* hydrochloric acid. The solution is heated at 100° for 2 hours and cooled, and the product is collected; yield 1.382 g. (81%), m.p. 173–174°. The amide is readily hydrolyzed (quantitatively) by dilute acid, but only very slowly by 2 *N* sodium hydroxide.

[1]W. Tschelinzeff and W. Schmidt, Ber., *62*, 2210 (1929).

[2]L. Claisen and J. Shadwell, Ber., *11*, 620 (1878).

[3]R. C. Thomas, Jr., C. H. Wang and B. E. Christensen, J. Am. Chem. Soc., *73*, 5914 (1951).

[4]P. C. Zamecnik, R. B. Loftfield, M. L. Stephenson and J. M. Steele, Cancer Research, *11*, 592 (1951).

[5]D. M. Hughes and J. C. Reid, J. Org. Chem., *14*, 516 (1949).

[6]D. P. Groth and G. A. LePage, J. Am. Chem. Soc., 77, 1681 (1955).
[7]B. Magasanik and H. E. Umbarger, J. Am. Chem. Soc., 72, 2308 (1950).
[8]H. Busch, Cancer Research, *15*, 365 (1955).
[9]D. P. Groth, G. A. LePage, C. Heidelberger and P. A. Stoesz, Cancer Research, *12*, 529 (1952).
[10]H. Busch, R. B. Hurlbert and V. R. Potter, J. Biol. Chem., *196*, 717 (1952).
[11]M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 210.
[12]C. H. Wang, R. F. Labbe, B. E. Christensen and V. H. Cheldelin, J. Biol. Chem., *197*, 645 (1952).
[13]E. H. Mosbach, E. F. Phares and S. F. Carson, J. Am. Chem. Soc., 73, 5477 (1951).
[14]R. M. Lemmon, Atomic Energy Commission Report, AECU-452; Nuc. Sci. Abstracts, 3, 1626 (1949).
[15]M. Calvin and R. M. Lemmon, J. Am. Chem. Soc., 69, 1232 (1947).
[16]R. O. Brady and S. Gurin, J. Biol. Chem., *186*, 461 (1950).
[17]E. Leete, L. Marion and I. D. Spenser, Can. J. Chem., 33, 405 (1955).
[18]D. I. Crandall and S. Gurin, J. Biol. Chem., *181*, 829 (1949).
[19]B. Schepartz and S. Gurin, *ibid.*, *180*, 663 (1949).
[20]R. G. Gould, A. B. Hastings, C. B. Anfinsen, I. N. Rosenberg, A. K. Solomon and Y. J. Topper, *ibid.*, *177*, 727 (1949).
[21]N. S. Radin, D. Rittenberg and D. Shemin, *ibid.*, *184*, 755 (1950).
[22]H. J. Strecker, H. G. Wood and L. O. Krampitz, *ibid.*, *182*, 525 (1950).
[23]H. R. V. Arnstein and R. Bentley, J. Chem. Soc., *1951*, 3436.
[24]J. M. Foster and C. A. Villee, J. Biol. Chem., *211*, 797 (1954).
[25]G. Milhaud, A. A. Benson and M. Calvin, Atomic Energy Commission Report, UCRL-2933; Nuc. Sci. Abstracts, 9, 4929 (1955).
[26]D. B. Sprinson and E. Chargaff, J. Biol. Chem., *164*, 417 (1946).

POTASSIUM PYRUVATE-3-C[14]

$$\begin{matrix} C^*H_2COOC_2H_5 \\ | \\ COCOOC_2H_5 \end{matrix} \xrightarrow{H_2SO_4} \begin{matrix} C^*H_2COOH \\ | \\ COCOOH \end{matrix} \xrightarrow[\Delta]{} C^*H_3COCOOH \xrightarrow{KOH} C^*H_3COCOOK$$

R. M. Lemmon, Atomic Energy Commission Report, AECU-452; Nuc. Sci. Abstracts, 3, 1626 (1949).

A. Procedure

Crude ethyl oxalacetate-3-C[14] is heated with 6 volumes of 20% sulfuric acid at 65–75° for 3 hours in a slow stream of nitrogen (Note 1). The acid solution is extracted with ether, the extract is dried and concentrated, and the residue is distilled (0.1 μ) into a trap cooled with liquid nitrogen (Note 2). A solution of the distilled pyruvic-3-C[14] acid in 5 volumes of methanol is treated with an ethanolic solution of potassium hydroxide (20% excess), and the mixture is diluted with 3–4 volumes of absolute ether. The salt is collected on a filter, washed with ether and dried; yield 11% based on sodium acetate (Note 3).

B. Notes

1. The course of the hydrolysis may be followed by observing the dissolution of the free acid formed from the insoluble ester. The course of the decarboxylation may be followed by passing the gas stream through a barium hydroxide bubbler.

2. Although the distillation flask is warmed ultimately to 90°, considerable nonvolatile radioactive organic residue is left, presumably polymer.

3. The 2,4-dinitrophenylhydrazone was formed with unchanged molar specific activity.

C. Other Preparations

The preparation of pyruvonitrile-3-C^{14}, pyruvamide-3-C^{14} and pyruvic-3-C^{14} acid by the method of Anker (see pyruvic-2-C^{14} acid) has been reported by Foster.[1]

Phenylpyruvic-3-C^{14} acid has been prepared by Brown[2] at the 5-mmole level by the Erlenmeyer azlactone synthesis.[3] Benzaldehyde-C^{14} is condensed with *N*-acetylglycine, and the 4-benzylidene-α-C^{14}-2-methyl-2-oxazolin-5-one is hydrolyzed to α-acetamidocinnamic-β-C^{14} acid, which is hydrolyzed in turn to phenylpyruvic-3-C^{14} acid. The crystalline product is collected, washed with ice water, dried at room temperature in a stream of dry air (1 hour) and used at once.

[1] J. M. Foster and C. A. Villee, J. Biol. Chem., *211*, 797 (1954).

[2] S. A. Brown and C. A. Neish, Can. J. Biochem. and Physiol., *33*, 948 (1955); *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 1, 519.

[3] *Organic Reactions*, Vol. III, Wiley, New York, 1946, p. 205.

SODIUM ACETOACETATE-1,3-C_2^{13}

$$CH_3C^*OOC_2H_5 \xrightarrow[2.\ HOAc]{1.\ (C_6H_5)_3CNa} CH_3C^*OCH_2C^*OOC_2H_5 \xrightarrow{NaOH}$$

(a)

$$CH_3C^*OCH_2C^*OONa$$

(b)

W. Sakami, W. E. Evans and S. Gurin, J. Am. Chem. Soc., *69*, 1110 (1947).

A. Procedure

(a) *Ethyl Acetoacetate-1,3-C_2^{13}.* The ethyl acetate-1-C^{13} prepared from 2.4 g. (29.3 mmoles) of fused sodium acetate-1-C^{13} is distilled (Note 1)

into an etheral solution of triphenylmethylsodium according to the procedure of Hudson,[1] which follows. The product is distilled in a slow stream of nitrogen; yield 0.55 g., 29% based on sodium acetate, or 44% based on the sodium acetate not recovered (Note 2).

An ether solution of triphenylmethylsodium is prepared[2] by shaking together 63 g. of triphenylchloromethane, 2,100 g. of 1% sodium amalgam and 1,500 ml. of absolute ether in a stoppered bottle under dry nitrogen. When a persistent blood-red color develops (15 minutes), shaking is stopped, the bottle is cooled with a wet towel, and the shaking is continued for 30 minutes. The sodium chloride is allowed to settle, and the ether solution is transferred to a nitrogen-filled flask by siphoning under nitrogen pressure; the yield is almost quantitative (Note 3). To the ether solution is added 16 g. of ethyl acetate, and the solution is shaken at room temperature; within one minute the deep red color changes to orange (Note 4). After 3 minutes, 20 ml. of glacial acetic acid is added, and the ether solution is washed with dilute sodium carbonate solution, dried with Drierite and concentrated by distillation at the water-bath through a short column. The residue is cooled, the precipitated triphenylmethane is filtered off, and the filtrate is fractionated, yielding a little ethyl acetate and 5 g. of product (43%), b.p. 73–76° (14 mm.).

(b) *Sodium Acetoacetate-1,3-*C_2^{13} (Note 5). The ester is hydrolyzed by standing for 6 hours with a slight excess of *N* sodium hydroxide. The excess alkali is neutralized, and the alcohol is removed by lyophilization. The yield (Note 6) is 91% based on ethyl acetoacetate (Note 7).

B. Notes

1. The ester is distilled with an oil-bath at 100–150° in nearly quantitative yield.

2. Sodium acetate is recovered to the extent of 35%.

3. The ether solution of triphenylmethylsodium is assayed by slowly running a 50-ml. aliquot into 25 ml. of water and extracting the ether solution with three 25-ml. portions of water. The combined aqueous solutions are boiled to expel ether, cooled and titrated to the end point of methyl red with standard acid.

4. Further addition of ethyl acetate produces no color change.

5. The procedure is according to Ljunggren.[3]

6. The product is analyzed by either the macro titrimetric or the micro colorimetric method described by Lorber.[4] Another method of analysis is described by Van Slyke.[5]

7. The isotope content of the product was 3.80 atoms per cent excess C^{13} compared to 3.88 for the sodium acetate-C^{13} starting material. The product is degraded to carbon dioxide and acetone by the procedure de-

scribed by Edson.[6] The carbon dioxide contained 7.60 atom per cent excess C^{13}, while the acetone contained 2.53 atom per cent excess.

C. Other Preparations

By the same procedures, starting with ethyl acetate-2-C^{14}, are prepared[7,8] ethyl acetoacetate-2,4-C_2^{14} and sodium acetoacetate-2,4-C_2^{14}

Sodium acetoacetate-1,3-C_2^{13} has been prepared by Weinhouse,[9] by condensing ethyl acetate-1-C^{13} in the presence of dry sodium ethoxide in a sealed tube at 108° for 16 hours.

[1]E. B. Hudson, Jr., R. H. Dick and C. R. Hauser, J. Am. Chem. Soc. *60*, 1960 (1938).

[2]C. R. Hauser and W. B. Renfrow, Jr., J. Am. Chem. Soc., *59*, 1823 (1937).

[3]G. Ljunggren, Biochem. Z., *145*, 422 (1924).

[4]L. Lorber, Biochem. Z., *181*, 366 (1927).

[5]D. D. Van Slyke, J. Biol. Chem., *83*, 415 (1929).

[6]N. L. Edson, Biochem. J., *29*, 2082 (1935); J. M. Buchanan, W. Sakami, S. Gurin and D. W. Wilson, J. Biol. Chem., *159*, 695 (1945).

[7]R. O. Brady and S. Gurin, J. Biol. Chem., *189*, 371 (1951).

[8]W. G. Brown, Jr., D. D. Chapman, H. R. Matheson, I. L. Chaikoff and W. G. Dauben, J. Biol. Chem., *209*, 537 (1954).

[9]S. Weinhouse, G. Medes and N. F. Floyd, J. Biol. Chem., *166*, 691 (1946).

OXALACETIC-4-C^{14} ACID
(2-Oxosuccinic-4-C^{14} Acid)

$$CH_3C^*OBr \xrightarrow[C_6H_5N(CH_3)_2]{(CH_3)_3COH} CH_3C^*OOC(CH_3)_3 \xrightarrow[KOC(CH_3)_3]{[-COOC(CH_3)_3]_2}$$

(a)

$$\begin{matrix} CH_2C^*OOC(CH_3)_3 \\ | \\ COCOOC(CH_3)_3 \end{matrix} \xrightarrow[p\text{-}CH_3C_6H_4SO_3H]{} \begin{matrix} CH_2C^*OOH \\ | \\ COCOOH \end{matrix} + CO_2 + CH_2{=}C(CH_3)_2$$

(b) (c)

C. Heidelberger and R. B. Hurlbert, J. Am. Chem. Soc., *72*, 4704 (1950).

A. Procedure

(a) *t-Butyl Acetate-1-C^{14}*. To an ice-cold mixture of 1.35 ml. of *t*-butyl alcohol, 1.95 ml. of distilled *N,N*-dimethylaniline and 5 ml. of absolute ether is added slowly 1.4 g. of acetyl-1-C^{14} bromide. The solu-

tion is refluxed for 1 hour and diluted with water; then the ether phase is separated, washed with water, alkali and water, and dried over sodium sulfate. The solvent is evaporated, dry toluene is added as a high-boiling chaser, and the product is collected by distillation at 80–95°; yield 0.69 g. (53%).

(b) *t-Butyl Oxalacetate-4-C*^{14}. To a solution of 0.24 g. of potassium in 7.5 ml. of *t*-butyl alcohol is added 1.28 g. of *t*-butyl oxalate[1] and the *t*-butyl acetate-1-C^{14}; then the solution is refluxed for 3 hours, during which time it solidifies gradually. The cooled mixture is treated with dilute sodium hydroxide, and the aqueous solution is washed with ether, acidified, and extracted with ether. The ether solution is dried over sodium sulfate and evaporated to dryness. The yield is 1.14 g. (79%) of an oily product, which is sufficiently pure for the next step (Note 1).

(c) *Oxalacetic-4-C*^{14} *Acid* (Note 2). A mixture of 1.00 g. of the crude *t*-butyl oxalacetate-4-C^{14}, 5 ml. of benzene and 0.020 g. of *p*-toluenesulfonic acid is refluxed until no further isobutene and carbon dioxide are evolved (1 hour). The product crystallizes and is collected; yield 0.487 g. (90%), m.p. 183–184° (Note 3).

B. Notes

1. Crystallization may be effected by evaporating a ligroin solution to dryness. The melting point of the pure compound, obtained by three recrystallizations from alcohol-water, is 90–91°.

2. The synthesis is new and is based on the method of Breslow[2] for synthesizing β-keto esters by an acid-catalyzed elimination of isobutene from a *t*-butyl ester. The yield by the usual hydrolysis of ethyl oxalacetate is only 9%, due to extensive decarboxylation, but Heidelberger and Hurlbert have developed a new method of hydrolysis which gives consistent yields of 30–35%. The isotopic procedure has been used by Weed.[3]

3. The over-all yield is 36% based on sodium acetate. The compound obtained by this method is actually hydroxyfumaric acid[4] (*trans*), whereas the compound obtained by the aqueous hydrolysis of the ethyl ester is hydroxymaleic acid (*cis*-enol form). The *trans*-form is converted to the *cis*-form, m.p. 152°, by dissolution in water and reisolation as quickly as possible.

The paper chromatographic characterization of and the determination of oxalacetic acid by the aniline citrate method[5] have been described by Kaltenbach.[6]

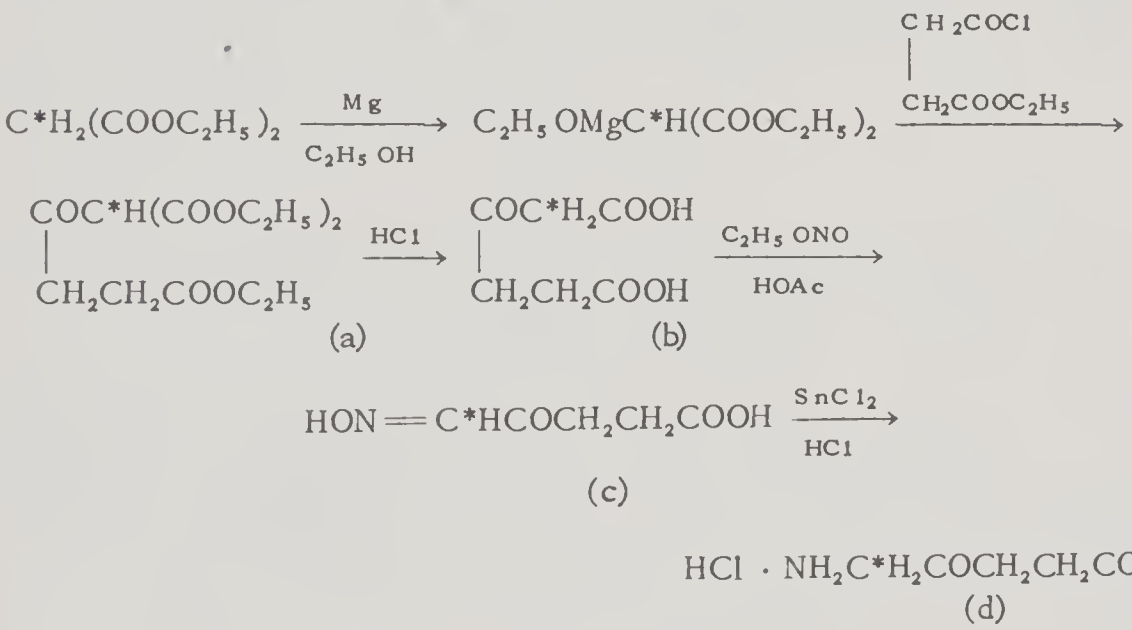
Studies of the C^{13}-isotope effect in the paramagnetic ion catalysis of decarboxylation of oxalacetic acid have been reported by Gelles.[7]

C. Other Preparations

In a similar manner, starting with acetyl-2-C^{14} bromide, are prepared:[8] *t*-butyl acetate-2-C^{14}, *t*-butyl oxalacetate-3-C^{14}, and oxalacetic-3-C^{14} acid (2-oxosuccinic-3-C^{14} acid).

[1]H. J. Backer and J. D. H. Homan, Rec. trav. chim., *58*, 1048 (1939).
[2]D. S. Breslow, E. Baumgarten and C. R. Hauser, J. Am. Chem. Soc., *66*, 1286 (1944).
[3]L. L. Weed and D. W. Wilson, J. Biol. Chem., *207*, 439 (1954).
[4]A. Wohl, Ber., *40*, 2282 (1907).
[5]N. L. Edson, Biochem. J., *29*, 2082 (1935).
[6]J. P. Kaltenbach and G. Kalnitsky, J. Biol. Chem., *192*, 629 (1951).
[7]E. Gelles and R. I. Reed, Nature, *176*, 1262 (1955).
[8]A. M. Delluva, Arch. Biochem., *45*, 443 (1953).

5-AMINOLEVULINIC-5-C^{14} ACID HYDROCHLORIDE
(5-Amino-4-oxovaleric-5-C^{14} Acid Hydrochloride)



D. Shemin, C. S. Russell and T. Abramsky, J. Biol. Chem., *215*, 613 (1955); D. Shemin and C. S. Russell, J. Am. Chem. Soc., *75*, 4873 (1953).

A. Procedure (Note 1)

(a) *Ethyl 2-Carbethoxy-3-oxohexanedioate-2-C^{14}.* Ethyl malonate-2-C^{14} (8 g.) is converted to ethyl ethoxymagnesiummalonate-2-C^{14} by the procedure of Lund and Voigt[1,2] then condensed with 9.3 g. of 3-carbethoxypropionyl chloride (Note 2) by the procedure of Reigel and Lilienfeld,[2] which follows.

The ether and most of the alcohol are removed from the ethoxymagnesiummalonic ester (1-mole level) by distillation under reduced pressure.

The syrupy residue is dissolved in 300 ml. of dry benzene, and the solvent is again distilled. To a solution of the residue in 300 ml. of dry benzene is added a solution of 1.15 moles of 3-carbethoxypropionyl chloride in 150 ml. of absolute ether at such a rate as to maintain vigorous refluxing. The mixture is refluxed for 2 hours, then hydrolyzed by the gradual addition of 30 ml. of sulfuric acid in 600 ml. of water. The aqueous layer is separated and extracted with ether. The combined extract is washed with dilute acid and water, then dried over sodium sulfate, concentrated and vacuum-distilled (Note 3).

(b) *3-Oxohexanedioic-2-C^{14} Acid.* The triester is hydrolyzed with concentrated hydrochloric acid at room temperature for 18 hours, according to the method of Eisner, Elvidge and Linstead,[3] which follows.

A mixture of 76.5 g. of ethyl 2-carbethoxy-3-oxohexanedioate and 200 ml. of concentrated hydrochloric acid is stored at room temperature for 36 hours. The solution is evaporated under reduced pressure at 30–35°, and the residue is stored overnight in a desiccator over potassium hydroxide to remove hydrochloric acid; yield 42 g. (100%), m.p. 115° (dec.) (Note 4).

(c) *5-Hydroxyiminolevulinic-5-C^{14} Acid* (Note 5). Into a suspension of the crude keto acid in 50 ml. of glacial acetic acid at 15–20° is passed an equimolar amount of ethyl nitrite (Note 6) during 20 minutes. The mixture is stored at room temperature for 1 hour; then the solution is evaporated under reduced pressure at 30–35°.

(d) *5-Aminolevulinic-5-C^{14} Acid Hydrochloride, (3-Glycyl-2-C^{14}-propionic Acid).* To an ice-cold solution of 25 g. of stannous chloride in 25 ml. of concentrated hydrochloric acid is added dropwise the oily crude 5-hydroxyiminolevulinic-5-C^{14} acid. The solution is stored in a refrigerator for 2 days, then diluted with 500 ml. of water and saturated with hydrogen sulfide. The combined filtrate and washings are evaporated under vacuum, the residue is dried over sodium hydroxide, and the crude product is recrystallized from absolute ethanol-ethyl acetate; yield 2.0–2.2 g., m.p. 149–151° (Note 7).

B. Notes

1. This synthetic method is one of three new ones developed by Shemin and Russel for preparing 5-aminolevulinic acid.

Another method designed as a basis for individual labeling of all five carbon atoms has been outlined by Neuberger and Scott.[4] Phthalimidoacetyl chloride is condensed with diethyl methyl ethanetricarboxylate, followed by ester exchange with benzyl alcohol, catalytic hydrogenolysis and hydrolysis of the 5-phthalimidolevulinic acid.

2. Directions are given by Riegel[2] for preparing the starting material by alcoholysis of succinic anhydride with absolute ethanol, followed by

halogenation of the ethyl hydrogen succinate with thionyl chloride; yield 94%, b.p. 110–115° (30 mm.).

3. The triester (73% yield based on 3-carbethoxypropionyl chloride)[3] distills at 144° (0.2 mm.)., n_D^{20} 1.4506.

4. 3-Oxohexanedioic acid, crystallized from acetone-chloroform, melts at 124–125°.

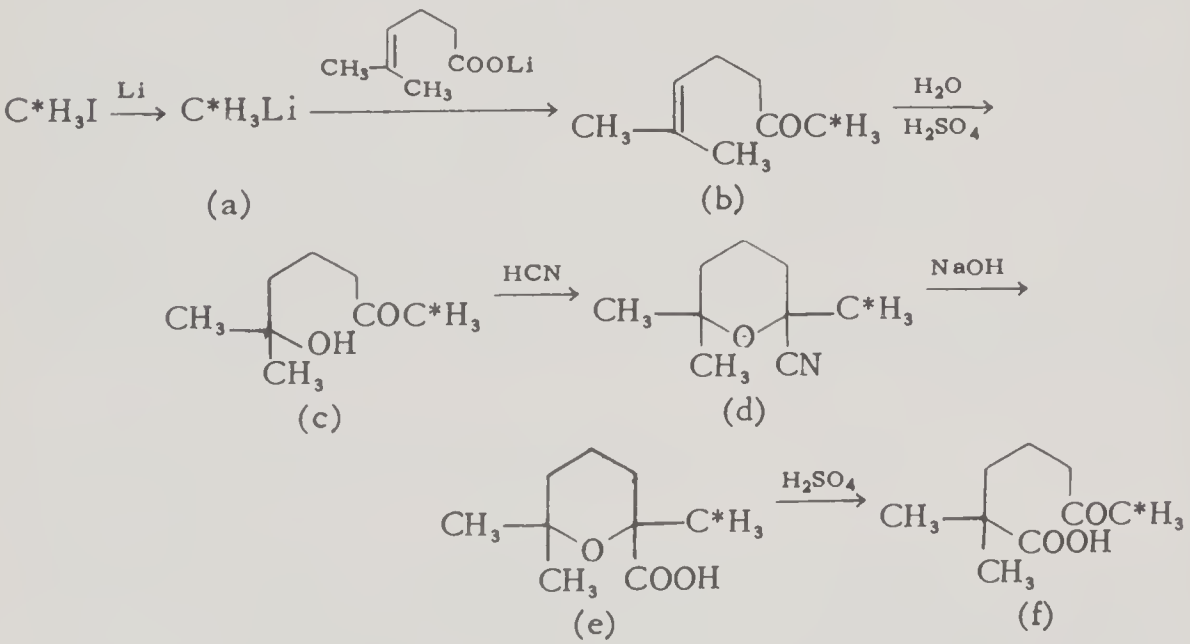
5. The preparation of α-hydroxyimino acids and α-amino acids is discussed by Barry and Hartung.[6]

6. The continuous generation of gaseous ethyl nitrite is described by Semon and Damerell.[5]

7. The product gives a positive ninhydrin test, reduces Benedict's solution in the cold, and gives a single spot (R_f 0.42–0.44) on an ascending paper chromatogram developed with phenol-water.

[1]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 594.
[2]B. Riegel and W. M. Lilienfeld, J. Am. Chem. Soc., *67*, 1273 (1945).
[3]U. Eisner, J. A. Elvidge and R. P. Linstead, J. Chem. Soc., *1950*, 2223.
[4]A. Neuberger and J. J. Scott, Nature, *172*, 1093 (1953).
[5]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 204
[6]R. H. Barry and W. H. Hartung, J. Org. Chem., *12*, 460 (1947).

2,2-DIMETHYL-6-OXOHEPTANOIC-7-C^{14} ACID
(C_1^{14}-Geronic Acid)



J. Meinwald, J. Am. Chem. Soc., 77, 1617 (1955).

A. Procedure (Note 1)

(a) *Methyllithium-C^{14}* (Note 2). To a stirred mixture of 2.5 g. of lithium chips and 50 ml. of absolute ether, under dry nitrogen, is added a solu-

tion of 17 g. of methyl-C^{14} iodide in 150 ml. of ether at a rate to maintain refluxing. The mixture is stirred for 1 hour and filtered into an addition funnel.

(b) *6-Methyl-5-hepten-2-one-1-C^{14}* (Note 3). To a stirred suspension of 16.5 g. of lithium 4-methyl-3-hexenoate[1] and 200 ml. of absolute ether under nitrogen is added slowly the solution of methyllithium-C^{14}. The mixture is refluxed for 6 hours, and the resulting clear solution is poured onto ice. The ether extract is washed, dried over magnesium sulfate and concentrated, and the product is distilled; yield 10.1 g. (74%), b.p. 81–84° (36 mm.) (Note 4).

(c) *6-Hydroxy-6-methyl-2-heptanone-1-C^{14}*. A mixture of 4.2 g. of 6-methyl-5-hepten-2-one-1-C^{14} and 45 g. of 35% sulfuric acid is shaken under nitrogen for 9 hours. The mixture is stored overnight in a refrigerator, then diluted with ice and water, and neutralized with sodium hydroxide, the temperature being maintained below 5° with an ice-salt bath. The mixture is continuously extracted with ether; the extract, dried over potassium carbonate, is concentrated; and the residue (4.9 g.) is distilled; yield 4.1 g. (85%), b.p. 100–102° (7 mm.).

(d) *Tetrahydro-2-methyl-C^{14}-6,6-dimethylpyran-2-carbonitrile*, (C_1^{14}-α-*Cinenonitrile*). A mixture of 4.0 g. of 6-hydroxy-6-methyl-2-heptanone-1-C^{14} and an equal volume of anhydrous hydrogen chloride is heated at 55° for 36 hours in a sealed tube. The tube is cooled with a Dry Ice-bath and opened, and the excess hydrogen cyanide is evaporated in a hood. The residue is dissolved in ether and washed with sodium hydroxide and water, the aqueous layers being reëxtracted with ether. The combined extract is dried over magnesium sulfate and distilled (Note 5); yield 2.77 (65%), b.p. 71–73° (10 mm.) (Note 4).

(e) *Tetrahydro-2-methyl-C^{14}-6,6-dimethylpyran-2-carboxylic Acid*, (C_1^{14}-α-*Cinenic Acid*). A mixture of 2.67 g. of the nitrile, 1 g. of potassium hydroxide, 2 ml. of methanol, 3 ml. of ethanol, and 3 ml. of water is refluxed for 24 hours, then treated with 1 g. of potassium hydroxide and 2 ml. of water, and the heating is continued for 24 hours (Note 6). The mixture is concentrated, cooled and washed with two portions of ether. The aqueous solution is acidified with hydrochloric acid, and the crude product is extracted into ether solution, which is washed with water, dried over magnesium sulfate and concentrated; crude yield 2.96 g., m.p. 81–82°. Purification is accomplished by dilution with 10.03 g. of pure synthetic carrier and recrystallization from 50 ml. of hot cyclohexane; yield 12.1 g., radiochemical yield 89% (Note 7). The over-all yield is 32% based on methyl iodide.

(f) *2,2-Dimethyl-6-oxoheptanoic-7-C^{14} Acid.* To 35 ml. of concentrated sulfuric acid at 5° is added slowly 5.97 g. of the powdered α-cinenic acid. The clear, colorless solution is stored for 21 hours under anhydrous conditions under dry nitrogen. The honey-colored solution is

poured onto ice, diluted with 400 ml. of water and continuously extracted with ether. The ether solution is extracted with sodium bicarbonate, and the alkaline solution is acidified and extracted with ether. The extract is dried over magnesium sulfate and evaporated, and the residue (3.39 g.) is distilled; yield 2.47 g. (41%), b.p. *ca* 135° (25 mm). (Note 8). Degradation studies confirm the location of isotope at C-7 (Note 9).

B. Notes

1. These preparations are incidental to a tracer study of the acid-catalyzed rearrangement of α-cinenic acid, essentially as described by Rupe.[2] The method of preparation is that of Rupe.[2,3]

2. The procedure is essentially that of van Dorp.[4] The reaction flask is equipped with an exit tube at its base containing a plug of glass wool and a stopcock for filtering the product solution. A large excess of lithium apparently does not increase the yield. Also see 2-(3,3,3-trichloro-2-hydroxypropyl-1-C^{14})quinoline.

3. The method is that of Gilman[5] for converting acids to the corresponding ketones by reaction of the lithium salt[1] with organolithium; see *N*-benzoylalanine-2-C^{13}.

4. The infrared spectrum is identical with that of an authentic sample.

5. A crystalline by-product from the hydration reaction remains in the distilling flask, m.p. 90–91°.

6. The evolution of ammonia ceases by 48 hours.

7. Pure α-cinenic acid melts at 83–84°.[3]

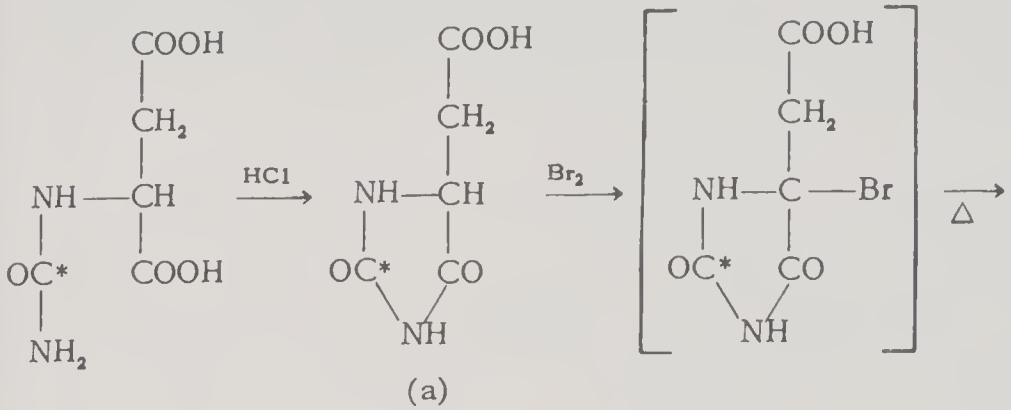
8. The product forms silky, colorless needles in a refrigerator, and the molar specific activity is the same as that of the starting material.

9. Directions are given by Meinwald for degrading the compound by the haloform reaction, with potassium iodide and Clorox, forming iodoform-C^{14} (75% yield) having the same molar specific activity as the precursor. The isomerization of α-cinenic acid, therefore, involves carboxyl rather than methyl migration.

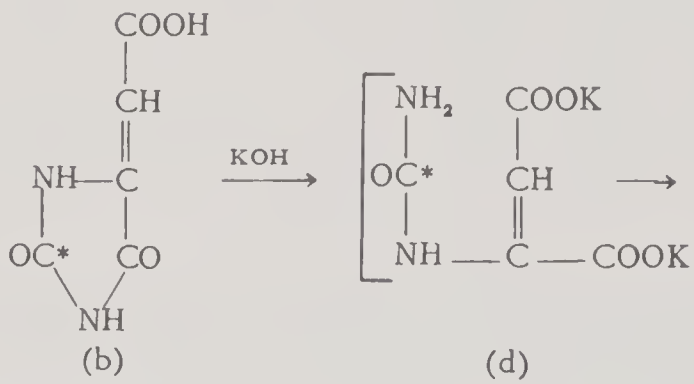
[1]R. P. Linstead and H. N. Rydon, J. Chem. Soc., *1933*, 580.
[2]H. Rupe and C. Liechtenhan, Ber., *41*, 1278 (1908).
[3]H. Rupe and P. Schlochoff, Ber., *38*, 1502 (1905).
[4]D. A. van Dorp and J. E. Ahrens, Rec. trav. chim., *65*, 338 (1946).
[5]H. Gilman and P. R. Van Ess, J. Am. Chem. Soc., *55*, 1258 (1933).

OROTIC-2-C^{14} ACID
(1,2,3,6-Tetrahydro-2,6-dioxo-4-pyrimidinecarboxylic-2-C^{14} Acid)

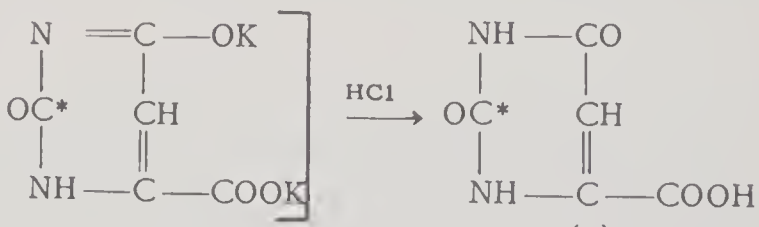
METHOD I



(a)



(b) (d)



(c)

L. I. Weed and D. W. Wilson, J. Biol. Chem., *189*, 435 (1951).

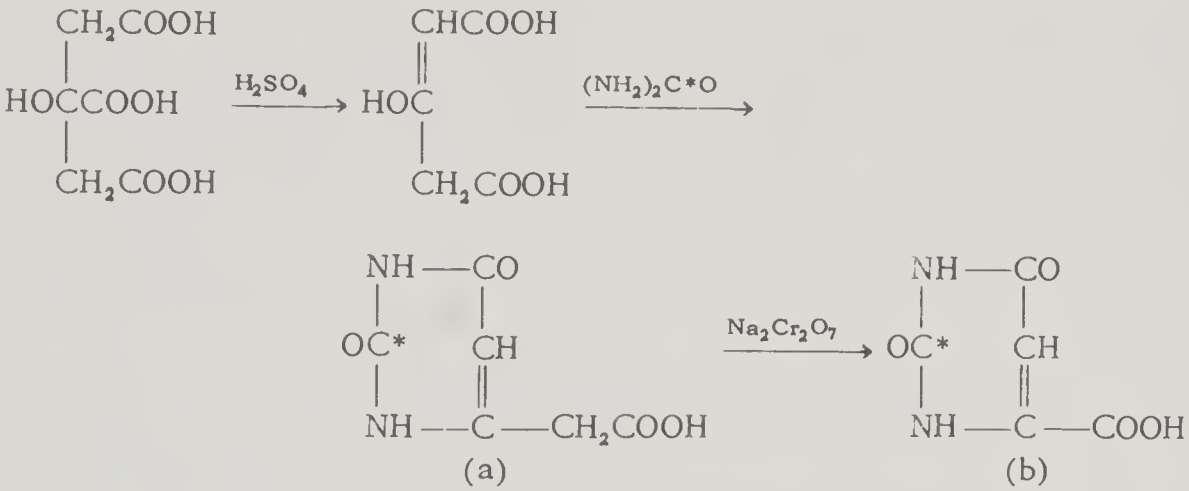
A. Procedure (Note 1)

(a) *5-Hydantoinacetic-2-C^{14} Acid.* A solution of 10.5 g. of *N*-carbamoyl-C^{14}-aspartic acid in 35 ml. of 20% hydrochloric acid is boiled nearly to dryness, and the residue is recrystallized from water; yield 7.9 g. (84%), m.p. 214-216°.

(b) *5-(Carboxymethylidene)hydantoin-2-C^{14}.* A mixture of 1.28 g. of bromine, 4 g. of the acid and 16 ml. of glacial acetic acid is heated at 100° with shaking for 1.5 hours in a sealed tube. The precipitate is filtered off, suspended in 50 ml. of boiling water for 20 minutes, then dissolved by adding sufficient water. The solution is cooled to crystallize the product; yield 2.92 g. (73%), m.p. above 400°.

(c) *Orotic-2-C^{14} Acid.* 5-(Carboxymethylidene) hydantoin-2-C^{14} (0.600 g.) is dissolved in 20 ml. of *M* potassium hydroxide in a constant temperature bath at 64°. After a 2-hour reaction period (Note 2), the solution is acidified with concentrated hydrochloric acid, and the mono-hydrated product is collected and recrystallized from water. The yield of anhydrous acid is 0.557 g. (93%), m.p. 343–345° (dec.) (sample immersed in a bath at 320°).

METHOD II



M. L. Tamayo, J. M. Gamboa and E. F. Alvarez, Anales real soc. españ. fís. y quím., *48B*, 889 (1952); through Chem. Abstracts, *48*, 1278 (1954).

A. Procedure

(a) *1,2,3,6-Tetrahydro-2,6-dioxo-4-pyrimidineacetic-2-C^{14} Acid* (Note 3). To 134 ml. of stirred fuming sulfuric acid (15% SO_3), cooled with a bath at −10°, is added 30 g. of powdered citric acid during 20 minutes, while keeping the temperature below 0° (Note 4), followed in turn by one-half of a mixture of 30 g. of urea and 30 g. of citric acid, then 0.00353 g. of urea-C^{14}, and finally the remainder of the mixture (Note 5). The mixture is heated to 75° for 30 minutes, then cooled, poured on to 400 g. of ice and stored in a refrigerator for 2 days. The product is collected and recrystallized from 150 ml. of hot water with slow cooling (Note 6); yield 15.2 g., m.p. 322°.

(b) *Orotic-2-C^{14} Acid.* A mixture of 14 g. of the pyrimidineacetic acid monohydrate, 25 g. of sodium dichromate, 196 ml. of water and 38.2 g. of concentrated sulfuric acid is heated to 55–60°, then the temperature is raised slowly to 75° for 1 hour. The mixture is cooled, and the crude product is collected, washed with cold water and recrystallized from hot water; yield 7.8 g. (monohydrate), m.p. 242° (Note 7).

B. Notes

1. The procedure is according to the method of Nyc and Mitchell,[1] which is given here.

2. The reaction time and possible rearrangement mechanism were established by determining the absorption spectrum of the mixture at time intervals. In contrast, three other 5,5-substituted hydantoins only opened the ring, forming an unsaturated hydantoic acid (d) in alkaline solution.

3. The procedure is an adaptation of the method of Hilbert[2] which is an improvement of the procedure of Davidson and Baudisch; see uracil-2-C^{14}.

4. The reaction is exothermic, and an appreciable amount of acetone dicarboxylic acid is formed if the temperature rises to 10°.

5. The temperature rises to 52°, and carbon monoxide is evolved.

6. The product crystallizes in the anhydrous condition (fine needles) upon rapid cooling, but as the monohydrate (stout prisms) upon slow cooling.

7. Directions are given by Tamayo for preparing the derivative 5,5-dibromobarbituric acid, m.p. 235°.

C. Other Preparations

The preparation of D-, L- and DL-5-hydantoinacetic-2-C^{14} acid (m.p. 214–217°) from the respective optically active precursor by Method I has been reported by Liebermann.[3] Purification was accomplished by ion exchange chromatography.

Orotic-6-C^{14} acid, (1,2,3,6-tetrahydro-2,6-dioxo-4-pyrimidinecarboxylic-6-C^{14} acid), has been prepared[4] from *N*-carbamoylaspartic-4-C^{14} acid by Method I, the intermediates being 5-hydantoinacetic-C^{14} acid and 5-(carboxy-C^{14}-methylidene)hydantoin. The bromination was accomplished with equimolecular amounts of reactants, and the sealed tube required periodic shaking during a 3-hour heating period.

The preparation of hydroörotic-2-C^{14} acid, (4,5-dihydroörotic-2-C^{14} acid), has been described by Cooper.[5] A solution of 0.050 g. of orotic-2-C^{14} acid in 40 ml. of glacial acetic acid was hydrogenated at room temperature and 50 p.s.i. over 0.070 g. of Adams catalyst for 20 hours in a Parr apparatus. The filtered solution was concentrated to 3 ml. under reduced pressure and diluted with 15 ml. of pentane. The product was separated by centrifuging, washed with pentane and recrystallized from 5 ml. of hot water by dilution with 3 ml. of alcohol; yield 0.048 g., m.p. 265–267°. This product is biologically active in contrast to other preparations.[6]

[1] J. F. Nyc and H. K. Mitchell, J. Am. Chem. Soc., 69, 1382, 674 (1947).
[2] G. E. Hilbert, J. Am. Chem. Soc., 54, 2076 (1932).

[3]I. Liebermann and A. Kornberg, J. Biol. Chem., *207*, 911 (1954); *212*, 909 (1955).

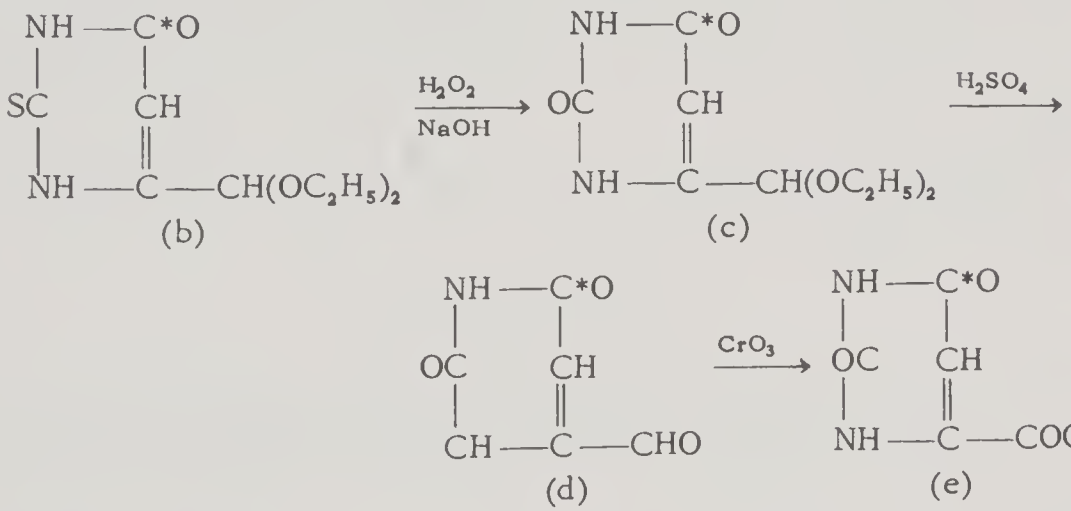
[4]D. W. Wilson, Nucleonics, 7 (4), 51 (1950).

[5]C. Cooper, R. Wu and D. W. Wilson, J. Biol. Chem., *216*, 37 (1955).

[6]M. Bachstez and G. Cavillini, Ber., *66*, 681 (1933).

OROTIC-6-C^{14} ACID

$$(C_2H_5O)_2CHCOOC_2H_5 \xrightarrow[NaH]{CH_3C^*OOC_2H_5} \underset{(a)}{\left[(C_2H_5O)_2CHCOCHC^*OOC_2H_5\right]}Na \xrightarrow{(NH_2)_2CS}$$



C. Heidelberger and R. B. Hurlbert, J. Am. Chem. Soc., *72*, 4704 (1950); Nucleonics, 7 (4), 51 (1950).

A. Procedure (Note 1)

(a) *Ethyl 4,4-Diethoxysodiumacetoacetate-1-C^{14}.* To a mixture of 0.220 g. (9.2 mmoles) of sodium hydride and 7 ml. of dry toluene at 90–100° is added 1.0 g. of ethyl diethoxyacetate (Note 2) in 2 ml. of toluene, followed by a mixture of 0.61 g. of ethyl diethoxyacetate, 2 ml. of toluene and the ethyl acetate-1-C^{14} prepared from 6.1 mmoles of sodium acetate-1-C^{14}. The mixture is heated at 100° until the hydride dissolves (2 hours); then the solvent is removed under reduced pressure.

(b) *1,2,3,6-Tetrahydro-6-oxo-2-thioxo-4-pyrimidinecarboxaldehyde-6-C^{14} Diethyl Acetal, (2-Thio-6-uracilcarboxaldehyde-4-C^{14} Diethyl Acetal).* A mixture of the crude ethyl 4,4-diethoxysodiumacetoacetate-1-C^{14}, 10 ml. of absolute alcohol and 0.700 g. (9.2 mmoles) of thiourea is refluxed for 3 hours. The solvent is distilled under vacuum, 10 ml. of water is added, and the mixture is boiled with 0.05 g. of Norit and filtered. The colored filtrate is cooled and acidified to pH 4, and the precipitated

product is collected, washed with cold water and dried; yield 0.975 g. (4.2 mmoles, 69% based on sodium acetate), m.p. 157–158°.

(c) *1,2,3,6-Tetrahydro-2,6-dioxo-4-pyrimidinecarboxaldehyde-6-C^{14} Diethyl Acetal, (6-Uracilcarboxaldehyde-4-C^{14}-Diethyl Acetal).* To a solution of the crude 2-thio-6-uracilcarboxaldehyde-4-C^{14} diethyl acetal in 7 ml. of water is added 3 ml. of 30% hydrogen peroxide. The solution is cooled in an ice-bath, treated slowly with 3.0 ml. of 5 *N* sodium hydroxide, then gradually warmed to room temperature and finally boiled to decompose the excess reagent. The mixture is cooled with an ice-bath and treated with 1.5 ml. of concentrated sulfuric acid to precipitate the product (Note 3).

(d) *1,2,3,6-Tetrahydro-2,6-dioxo-4-pyrimidinecarboxaldehyde-6-C^{14}, (6-Uracilcarboxaldehyde-4-C^{14}).* The acid mixture containing the acetal is boiled for 2 minutes, to effect hydrolysis and expel sulfur dioxide, and then is cooled in an ice-bath (Note 4).

(e) *Orotic-6-C^{14} Acid, (6-Uracilcarboxylic-4-C^{14} Acid).* To the ice-cold acid mixture containing the 6-uracilcarboxaldehyde-4-C^{14} is added 1.20 g. (12 mmoles) of chromic acid, followed by 1.5 ml. of concentrated sulfuric acid. The solution is stored at room temperature for 2 hours, then heated to 85° for 15 minutes and cooled in ice. The product is collected, washed with cold water and recrystallized from dilute hydrochloric acid (Norit) and from hot water. The yield of the monohydrate is 0.475 g. (65% based on thiouracil acetal), m.p. 345° (dec.) (Note 5). Paper chromatography (Note 6) shows that the product is more than 99% homogeneous. The molar specific activity is the same as that of the sodium acetate-1-C^{14} starting material.

B. Notes

1. The procedure is a considerably modified version of the method of Johnson and Schroeder,[1] giving considerably improved yields. The original one-step oxidation procedure gives poor yields on a small scale.

2. The ester is prepared from dichloroacetic acid and sodium ethoxide by the method of Johnson and Cretcher.[2] Several precautions are given by Johnson and Schroeder.[1]

3. 6-Uracilcarboxaldehyde diethyl acetal may be collected, washed with a little cold water and recrystallized from hot water. The yield is nearly quantitative, m.p. 179°.

4. Prolonged boiling results in a deepening color. 6-Uracilcarboxaldehyde crystallizes upon standing and may be collected, washed with cold water and recrystallized from hot water, or dilute hydrochloric acid. The yield is practically quantitative, m.p. 273–275° (char without melting). The one water of crystallization is lost at 120°. The phenylhydrazone decomposes at 330°.

5. The anhydrous acid, formed at 130°, exhibits a neutralization equivalent of 158 (calc. 156). Acetic acid precipitates the corresponding salts from potassium hydroxide or ammonium hydroxide solution.

6. The two solvent systems used are phenol-water and butanol-propionic acid-water.

C. Other Preparations

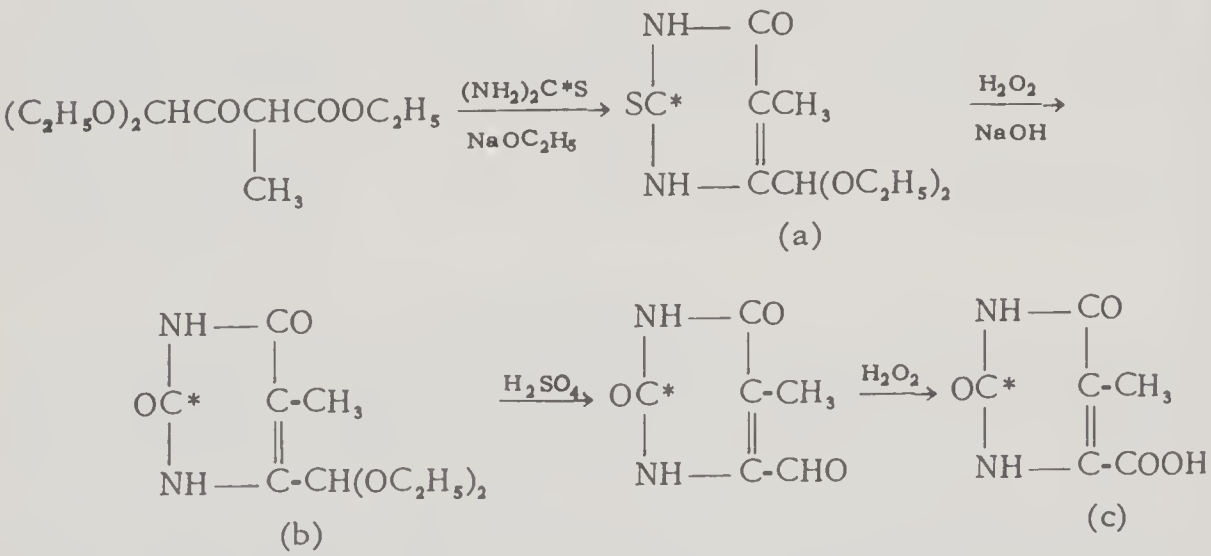
Orotic-6-C^{14} acid has been prepared from aspartic-4-C^{14} acid by Wilson[3] (40% yield). The method is a slight modification of that described by Nyc and Mitchell; see orotic-2-C^{14} acid, Note 1. The intermediates are: *N*-carbamoylaspartic-4-C^{14} acid, 5-hydantoinacetic-1-C^{14} acid and 5-(carboxy-C^{14}-methylidene)hydantoin.

[1]T. B. Johnson and E. F. Schroeder, J. Am. Chem. Soc., *53*, 1989 (1931).

[2]T. B. Johnson and L. H. Cretcher, Jr., *ibid.*, *37*, 2147 (1915); J. Biol. Chem. *26*, 29 (1916).

[3]D. W. Wilson, Nucleonics, 7 (4), 51 (1950); M. Edmonds, A. M. Delluva and D. W. Wilson, J. Biol. Chem., *197*, 251 (1952).

5-METHYLOROTIC-2-C^{14} ACID



W. L. Holmes and W. H. Prusoff, J. Biol. Chem., *206*, 817 (1954).

A. Procedure (Note 1)

(a) *1,2,3,6-Tetrahydro-5-methyl-6-oxo-2-thioxo-4-pyrimidinecarboxaldehyde Diethyl Acetal, (2-Thio-6-thyminecarboxaldehyde-2-C^{14} Diethyl Acetal).* To a hot solution of 1.4 mmoles of thiourea-C^{14} in a solution of 1.52 mmoles of sodium in 10 ml. of absolute ethanol is added 0.6 g. (2.4 mmoles) of ethyl 4,4-diethoxy-2-methylacetoacetate (Note 2), and the

mixture is refluxed for 6 hours under anhydrous conditions. The solvent is evaporated, and the residue is heated for 5 minutes at 100° with 15 ml. of water containing 0.010 g. of Norit. The filtered solution is evaporated to 2 ml., then cooled to 5°, and the pH is adusted to 6.0 by the dropwise addition of 3*N* hydrochloric acid with stirring. The product is crystallized at 0° and separated by centrifuging; yield 60–75%, m.p. 107–110°.

(b) *1,2,3,6-Tetrahydro-5-methyl-2,6-dioxo-4-pyrimidinecarboxaldehyde-2-C^{14} Diethyl Acetal, (6-Thyminecarboxaldehyde-2-C^{14} Diethyl Acetal).* To an ice-cooled mixture of the 2-thio-6-thyminecarboxaldehyde-2-C^{14} diethyl acetal, 1.0 ml. of water and 2 ml. of 30% hydrogen peroxide is added slowly 1.0 ml. of 4 *N* sodium hydroxide. The mixture is warmed to room temperature during 30 minutes, then cooled with an ice-bath and acidified with 0.4 ml. of concentrated sulfuric acid to precipitate the product.

(c) *5-Methylorotic-2-C^{14} Acid, (6-Thyminecarboxylic-2-C^{14} Acid).* The precipitated acetal is heated in the same reaction mixture for 25 minutes on a steam-bath to effect hydrolysis and oxidation (Note 3). The mixture is stored in a refrigerator for several hours to crystallize. The product is collected by centrifuging and recrystallized from dilute hydrochloric acid, and from 95% alcohol which is 0.5 *N* in hydrochloric acid; yield 0.090 g. (0.53 mmole), m.p. 310–311° (dec.) (Note 4). Purity is demonstrated by paper chromatography with two solvent systems (Note 5), and radioautography.

B. Notes

1. The procedure is a modification of the method of Johnson;[1] see thymine-2-C^{14} and the similar synthesis of orotic-6-C^{14} acid by Heidelberger and Hurlbert. The chromic acid oxidation of the aldehyde gave low yields in this case.

2. The acetal is prepared by the procedure of Johnson and Critcher.[2] Ethyl diethoxyacetate, prepared from dichloroacetic acid, is condensed with ethyl propionate in the presence of sodium.

3. Acid peroxide gives better results than chromic acid, acid (or alkaline) permanganate, nitric acid, or alkaline peroxide.

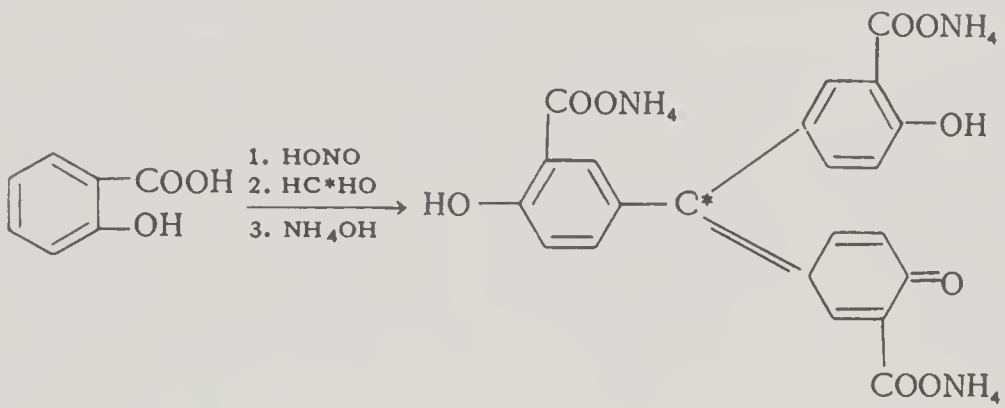
4. The neutralization equivalent agrees with the calculated value, 170. The ultraviolet absorption spectrum in 0.1 *N* potassium hydroxide agrees with that of an authentic sample.

5. 1-Butanol saturated with 0.1 *N* hydrochloric acid; descending, R_f 0.52. Isopentyl alcohol-5% disodium phosphate; ascending R_f 0.70. The same R_f values are obtained with an authentic sample.

[1] T. B. Johnson and L. H. Critcher, Jr., J. Biol. Chem., 26, 29 (1916).
[2] T. B. Johnson and L. H. Critcher, Jr., J. Am. Chem. Soc., 37, 2144 (1915).

4-[Bis(3-CARBOXY-4-HYDROXYPHENYL)METHYLENE-C^{14}]-2-CARBOXY-2,5-CYCLOHEXADIEN-1-ONE AMMONIUM SALT

(Ammonium C_1^{14}-Aurintricarboxylate) (C_1^{14}-Aluminon)



J. Schubert, M. R. White and A. Lindenbaum, J. Biol. Chem., *196*, 279 (1952).

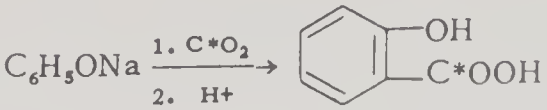
A. Procedure

The procedure is that described by Welcher,[1] which follows, suitably modified to permit use of a closed system.

To 44 ml. of concentrated sulfuric acid is added slowly 4 g. of sodium nitrite with stirring and avoidance of excessive evolution of oxides of nitrogen. To the stirred mixture at 10° is added 12 g. of salicylic acid during 5–10 minutes. To the stirred mixture is added dropwise 3.5 ml. of 37% formaldehyde solution, while the temperature is maintained at 3–5°. Stirring is continued at 5-minute intervals for 1 hour; then the mixture is stored in the cold for 20 hours and finally poured slowly into 2 l. of cold water. After standing for 1 hour, the precipitate is collected on a funnel and washed with three portions of water. The black solid is boiled for 2–3 minutes with a solution of 50 ml. of concentrated hydrochloric acid in 1 l. of water, then allowed to settle for 10 minutes and washed by decantation with three portions of water. The mass is pulverized, and the acid treatment and washing are repeated. The residue is dissolved in an excess of ammonium hydroxide, and the filtered solution is evaporated to dryness on a steam-bath.

[1]F. J. Welcher, *Organic Analytical Reagents*, Vol. 2, D. Van Nostrand, New York, 1947, p. 94; *Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 54; P. N. Rabinovitch and V. L. Pridorogin, Khim.-Farm. Prom., *1933*, 271; Chem. Abstracts, *28*, 3725 (1934); D. A. Holaday, J. Am. Chem. Soc., *62*, 989 (1940).

SALICYLIC-C^{14} ACID



H. G. Mandel and P. K. Smith, J. Am. Pharm. Assoc., Sci. Ed., 39, 479 (1950).

A. Procedure (Note 1)

The dried carbon-C^{14} dioxide prepared from 10.0 mmoles of barium carbonate-C^{14} (Note 2) is distilled, by cooling with liquid nitrogen, into a heavy-walled glass pressure flask, half-filled with glass beads (Note 3) and containing 2.5 g. (22 mmoles) of dried, pulverized sodium phenoxide (Note 4). The flask is sealed off, warmed to room temperature on a shaking machine, then heated for 40 hours in a sand-bath at 140° with occasional shaking. The mixture is dissolved in 100 ml. of water and filtered, and the product is precipitated by acidification with concentrated hydrochloric acid; yield 0.830 g. The mother liquor is extracted with ethylene dichloride, which is in turn extracted with a saturated solution of sodium carbonate (Note 5). A second crop of the acid is precipitated by acidification of the alkaline extract, making the total yield 0.94 g. (68%). Purification by treatment with charcoal and crystallization from water yield colorless needles, m.p. 157–158°. Paper chromatography and radioautography indicate the presence of only one isotopic compound (Note 6).

B. Notes

1. The procedure is an adaptation of the Schmitt modification of the Kolbe reaction for preparing phenolic carboxylic acids.[1]

2. Concentrated sulfuric acid is added slowly to 2.0 g. of barium carbonate-C^{14} under nitrogen at 600 mm., and the carbon dioxide is passed through a Drierite tube and a spiral trap cooled with a bath at –30°. The acid mixture then is warmed to effect solution, and the system is swept with a stream of nitrogen.

3. The beads increase the reaction surface area and facilitate mixing.

4. Distilled phenol (8.0 g., 84 mmoles) is added to a solution of 2.0 g. (87 mmoles) of sodium in 30 ml. of absolute alcohol, and the solution is refluxed for 30 minutes. The alcohol is distilled, and the residue is dried thoroughly for 24 hours in a vacuum desiccator, then powdered in a mortar.

5. The unreacted phenol remains in the organic solvent.

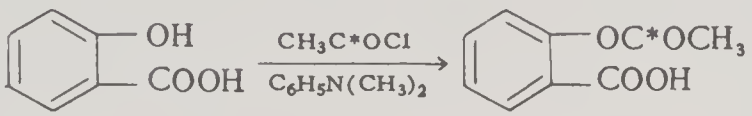
6. The compound is chromatographed on Whatman paper No. 1 in a solvent system of water-70% nitric acid (140:1,v/v); R_f 0.62.

C. Other Preparations

Salicylic-C^{14} acid has been prepared[2] from sodium phenoxide and carbon-C^{14} dioxide by a modification of the method of Schmitt;[1] yield 7.0%, m.p. 158–159° from water. The unreacted carbon dioxide was readily recovered.

[1]R. Schmitt, J. prakt. Chem., *31*, 397 (1885).
[2]R. W. Schayer, Arch. Biochem., *28*, 371 (1950).

ACETYL-1-C^{14}-SALICYLIC ACID
(C_1^{14}-Aspirin)



J. W. Clappison, J. P. Hummel, W. D. Paul and J. I. Routh, J. Am. Pharm. Assoc., Sci. Ed., *40*, 532 (1951).

A. Procedure

To a stirred solution of 9.2 g. of dry salicylic acid, 10 ml. of redistilled *N,N*-dimethylaniline and 50 ml. of absolute ether at 0° is added 51.7 mmoles of acetyl-1-C^{14} chloride by distillation. The mixture is stirred for 15 minutes; then the ether layer is decanted, and the insoluble *N,N* dimethylaniline hydrochloride is washed with three portions of ether. The combined solution is dried over sodium sulfate and concentrated to 20 ml., then is diluted with an excess of low-boiling petroleum ether and stored overnight at −15° to crystallize the product. The yield, after recrystallization from ether-petroleum ether, is 7.6 g. (69% based on sodium acetate-1-C^{14}).

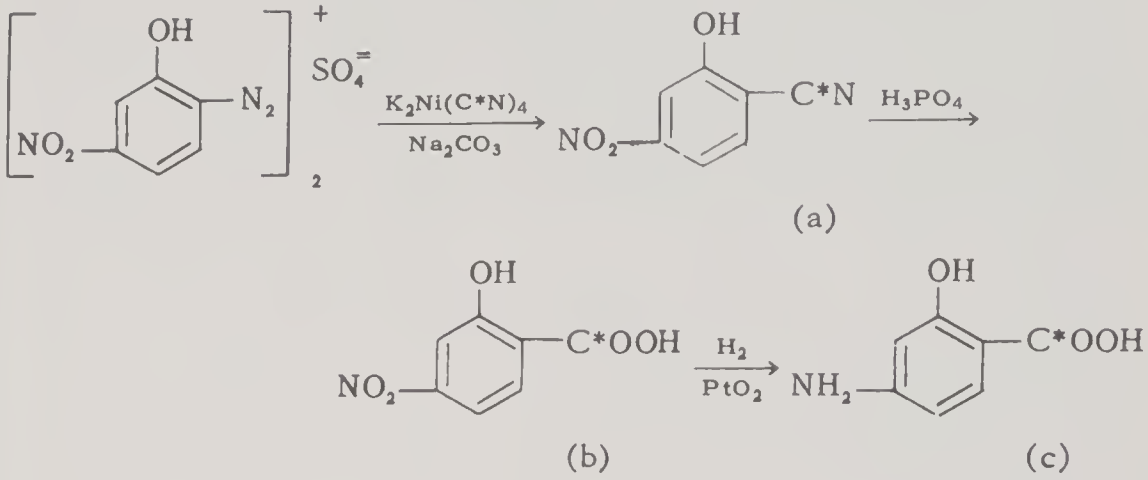
B. Other Preparations

Mandel[1] has prepared acetyl-1-C^{14}-salicylic acid, in 32% yield based on sodium acetate-1-C^{14}, by reacting bis(acetic-1-C^{14}) anhydride with salicylic acid according to the method of Vogel.[2] The acetic-1-C^{14} acid formed was recovered almost quantitatively. A 10% additional radiochemical yield was obtained by recrystallizing carrier aspirin from the mother liquors. Recrystallization from hot dilute alcohol leads to deacetylation. Paper chromatography in a system of water-70% nitric acid (140 : 1, v/v) showed the presence of only one radioactive compound.

[1]H. G. Mandel, The George Washington University School of Medicine, Washington, D. C., private communication.
[2]A. I. Vogel, *A Textbook of Practical Organic Chemistry, Including Qualitative Organic Analysis*, Longmans, London, 1948, p. 862.

4-AMINOSALICYLIC-C^{14} ACID

METHOD I



L. Clark, A. Heller and L. J. Roth, Department of Pharmacology, University of Chicago; J. Am. Pharm. Assoc., Sci. Ed., *44*, 328 (1955).

A. Procedure (Note 1)

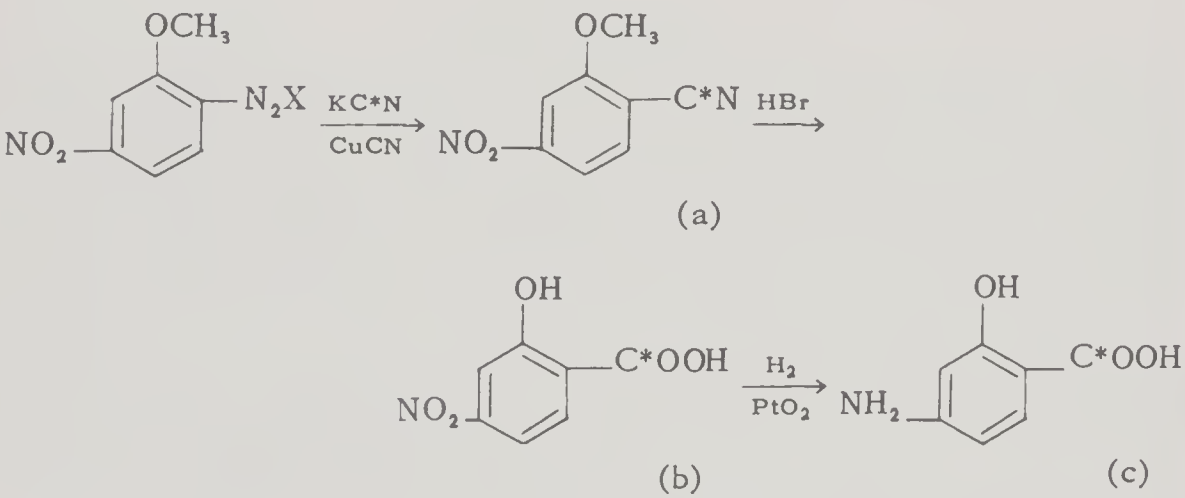
(a) *4-Nitrosalicylonitrile-C^{14}* (Note 2). To a stirred solution of 50 mmoles of potassium cyanide-C^{14} in 6 ml. of water, in a Waring Blendor (Note 3), is added 2.88 g. (11 mmoles) of hydrated nickel sulfate, followed by 20 g. of anhydrous sodium carbonate in 20 ml. of water. To the mixture, maintained below 30° and stirred as rapidly as possible with control of splashing, is added over a period of about 2 hours a filtered solution of 2-hydroxy-4-nitrobenzenediazonium sulfate, and additional sodium carbonate as required to keep the mixture weakly alkaline. The diazonium salt solution is prepared by adding slowly a solution of 1.74 g. (11.3 mmoles) of 2-amino-5-nitrophenol (Note 4) in 20 ml. of glacial acetic acid to a stirred solution of 1.08 g. (12.66 mmoles) of potassium nitrite in 6.4 ml. of concentrated sulfuric acid, below 20° (Note 5). Following the addition of the diazonium solution, the stirred mixture is heated for 30 minutes at 90° in round-bottomed flask, then cooled and adjusted to pH 6. The crystalline precipitate is collected and extracted with hot methanol, and the filtrate is evaporated to dryness. Purification is accomplished by vacuum sublimation; yield 0.794 g. (43% based on the amine), m.p. 160–161° (Note 6).

(b) *4-Nitrosalicylic-C^{14} Acid.* A mixture of 0.794 g. of 4-nitrosalicylonitrile-C^{14} and 3 ml. of 100% phosphoric acid is heated at 155–160° for 4 hours (Note 7), then cooled in an ice-bath and basified with 6 *N* potassium hydroxide. After adjustment of pH 6.5 with 2 *N* sulfuric acid, the solution is continuously extracted with ether for 2 hours. The aqueous solution is acidified to Congo red and extracted continuously

with ether (Note 8). The combined residues, left by ether removal, are recrystallized from boiling water which has been acidified with hydrochloric acid; yield 0.598 g. (70%), m.p. 234–235°.

(c) *4-Aminosalicylic-C^{14} Acid.* The 4-nitrosalicylic-C^{14} acid (0.598 g., 3.27 mmoles) is hydrogenated in 50 ml. of absolute alcohol solution at ordinary pressure and temperature over platinum oxide catalyst. The hydrogenation is discontinued 30 minutes after the uptake ceases (approximately 100%), and the catalyst is filtered off under nitrogen (Note 9). The filtrate is evaporated to dryness, and the residue is dissolved in a minimal volume of methanol at room temperature and decolorized with carbon; then the filtrate is diluted with five volumes of ice water to crystallize the product (Note 10). The yield of colorless needles is 0.400 g. (62%), m.p. 147–148° (dec.).

METHOD II



A. Wacker, H. Grisebach, A. Trebst, M. Ebert and F. Weygand, Angew. Chem., *66*, 712 (1954).

A. Procedure

(a) *4-Nitro-o-anisonitrile-C^{14}, (2-Methoxy-4-nitrobenzonitrile-C^{14}).* The diazonium salt solution prepared from 1.2 g. (6.75 mmoles) of 2-methoxy-4-nitroaniline is neutralized with calcium carbonate, filtered and added to a solution of 0.325 g. (5 mmoles) of potassium cyanide-C^{14} and 0.150 g. (1.67 mmoles) of cuprous cyanide in 10 ml. of water at 40°. The mixture is heated at 40° for 1 hour; then the crude product is collected and washed with water. Purification is accomplished by dissolution in benzene, chromatography on aluminum oxide and crystallization from acetone- water; yield 0.995 g. (84%), m.p. 178°.

(b) *4-Nitrosalicylic-C^{14} Acid.* A mixture of the nitrile, 16 ml. of glacial acetic acid and 22 ml. of constant-boiling hydrobromic acid is refluxed for 6 hours, then cooled, and the product is collected and washed with a little acetic acid. Purification is accomplished by vacuum sublimation (oil pump) at 160°; yield 0.620 g. (60%), m.p. 230–234°.

(c) *4-Aminosalicylic-C^{14} Acid.* The 4-nitrosalicylic-C^{14} acid is hydrogenated as described in Method I; yield 0.500 g., 49% based on potassium and copper cyanides. Paper chromatography shows that there are no isotopic impurities.

B. Notes

1. The preparation of 4-aminosalicylic acid from *m*-aminophenol by the Kolbe synthesis (see salicylic-C^{14} acid) results in the formation of about 9% 4-amino-6-hydroxyisophthalic acid,[1] which is not readily separable.

2. The procedure is a modification of the modified Sandmeyer reaction described by Storrie.[2]

3. As conventional stirring procedures are inadequate, the reaction flask consists of a Waring Blendor equipped with a 500-ml. screw-cap jar, fitted with holes for a thermometer and for the introduction of reactants. Stirring speed is controlled with a variable transformer.

4. Directions are given by Clark for acetylating *o*-aminophenol, nitrating the 2′-acetoxyacetanilide formed, separating the 4′-nitro isomer from the 6′-nitro derivative by mechanical means,[3] then hydrolyzing the ester group in alkali and the amide group in acid to form the starting material, m.p. 201–202°.

5. The finely powdered nitrite is added to the cooled acid with vigorous stirring, the temperature is raised to 70° to effect solution, and then the solution is cooled to room temperature and filtered from sodium bisulfate. Reversal of the order of addition precipitates the sulfate of the amine, greatly retarding the diazotization.[4]

6. The best yield in trial runs was 65%. The yellow powder may be recrystallized from aqueous methanol as pale yellow needles. *O*-Acetyl-4-nitrosalicylonitrile melts at 100–101°.

7. The procedure is based on the method of Berger.[5]

8. The receiver is changed at 2-hour intervals, and the extraction is continued as long as an appreciable residue is left upon evaporation of the ether.

9. Solutions of the product, in contact with active catalyst, darken rapidly on exposure to air.

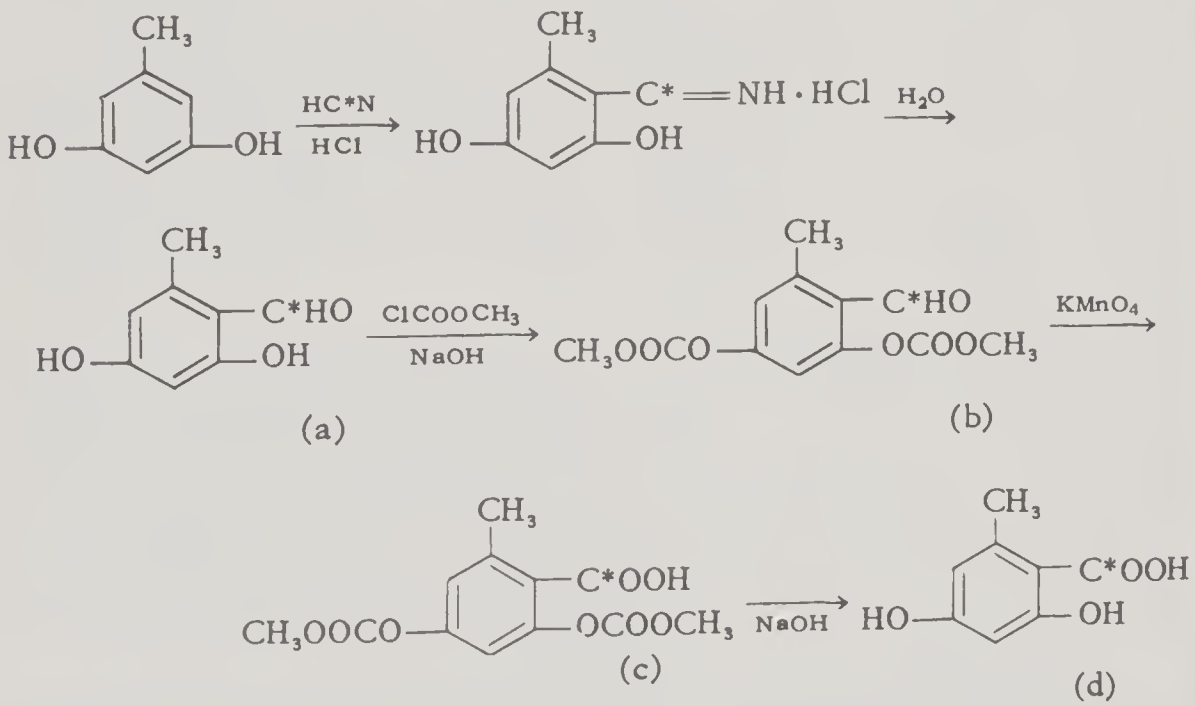
10. Aqueous solutions of 4-aminosalicylic acid, or its hydrochloride (m.p. 224° dec.), are decarboxylated above 80°. A trial preparation was converted by the Sandmeyer reaction to 4-chlorosalicylic acid, m.p. 207–209°.

C. Other Preparations

The preparation of 4-aminosalicylic-C^{14} acid by the Kolbe synthesis (Note 1) has been reported by Lormand.[6] A mixture of 1.0 g. of *m*-aminophenol, 4.0 g. of ammonium carbonate-C^{14} and 2.0 ml. of water is heated for 4 hours at 105° in a sealed tube (30–40 kg. of pressure in an autoclave). The mixture is heated for 1 hour in a beaker at 50–60°; then a filtered solution is adjusted to pH 3 with hydrochloric acid; yield 1.196 g.

[1]H. C. Beyerman and G. Alberda, Rec. trav. chim., *69*, 1021 (1950).
[2]F. R. Storrie, J. Chem. Soc., *1937*, 1746.
[3]H. H. Hodgson and J. Walker, *ibid*, *1933*, 1620.
[4]C. K. Ingold and E. H. Ingold, *ibid*, *1926*, 1320.
[5]G. Berger and S. C. J. Olivier, Rec. trav. chim., *46*, 600 (1927).
[6]C. Lormand, J. Desbordes, P. Bonet-Moury and A. Deysine, Ann. pharm. franc., *11*, 578 (1953).

o-ORSELLINIC-C^{14} ACID



R. O. Brady, J. Rabinowitz, J. Van Baalen and S. Gurin, J. Biol. Chem., *193*, 137 (1951).

A. Procedure

(a) o-*Orsellinaldehyde-*C^{14}, (*4,6-Dihydroxy-*o*-tolualdehyde-*C^{14}). Anhydrous orcinol is condensed with anhydrous hydrogen cyanide-C^{14} in

the presence of hydrogen chloride according to the procedure of Gattermann and Köbner,[1] which follows (Note 1).

Under a reflux condenser is passed a stream of dry hydrogen chloride through a solution of 12 g. of orcinol, 3.5 g. of anhydrous hydrogen cyanide and 30 g. of absolute ether until the crystalline aldimine hydrochloride is completely precipitated. The mixture is stored for a while; then the supernatant solution is removed, and the solid is washed with ether. The residue is dissolved in about 40 g. of boiling water, and the filtered solution is cooled to crystallize the product. The yield of recrystallized aldehyde is 13.5 g., m.p. 180°.

(b) *4,6-Bis(methoxycarbonyloxy)-o-tolualdehyde-C*^{14}, [*2-Formyl-C*^{14}*-3-methyl-1,5-phenylene Bis(methyl Carbonate)*]. The hydroxyl groups of *o*-orsellinaldehyde-C^{14} are blocked by the method of Hoesch,[2] which follows (Note 2).

To a solution of 7.6 g. of *o*-orsellinaldehyde in 30 ml. of acetone is added 4.7 g. of methyl chloroformate followed gradually, with ice-bath cooling and vigorous shaking, by 50 ml. of *N* sodium hydroxide. When the monocarbomethoxy-product (Note 3) begins to precipitate, the mixture is diluted with 40 ml. of acetone, cooled, and again treated with the same amount of methyl chloroformate and alkali. The rather pure product is precipitated in nearly quantitative yield by dilution of the mixture with an equal volume of water (Note 4). Purification may be achieved by recrystallization from ligroin or dilute acetone, m.p. 84–85° (Note 5).

(c) *4,6-Bis(methoxycarbonyloxy)-o-toluic-C*^{14} *Acid,* (*2-Carboxy-C*^{14}*-3-methyl-1,5-phenylene Dimethyl Carbonate*). The aldehyde group of "dicarbomethoxy-*o*-orsellinaldehyde-C^{14}" is oxidized by the method of Hoesch,[2] which follows.

To a stirred solution of 12 g. of "dicarbomethoxy-*o*-orsellinaldehyde" in 100 ml. of acetone, maintained at 40–45°, is added gradually a solution of 12 g. of potassium permanganate in 200 ml. of water. After 30–45 minutes, the ice-cooled suspension of manganese dioxide is treated with sulfurous acid, and the colorless crystalline product is collected and recrystallized from ethyl acetate and ligroin; yield 85%, m.p. 133° (cor.) (dec.) (Note 4).

(d) *o-Orsellinic-C*^{14} *Acid,* (*4,6-Dihydroxy-o-toluic-C*^{14} *Acid*). The dicarbomethoxy derivative is hydrolyzed according to the procedure of Hoesch,[2] which follows. The acid melts at 173–175° (dec.).

A mixture of 7.2 g. of "dicarbomethoxy-*o*-orsellinic acid" and five equivalents of *N* sodium hydroxide is stored at room temperature for 1.5 hours. The product is precipitated by acidification, collected and recrystallized from dilute alcohol. The yield of the monohydrate is 3.7 g. (78%), m.p. 173–176° (dec.) (Note 6).

B. Notes

1. The aluminum chloride or zinc chloride catalyst, usually used in the Gatermann method[3] for synthesizing aromatic aldehydes, is not neccessary with the active polyhydroxy benzenes. Adams and Levine[4] have prepared *o*-orsellinaldehyde in 85% yield, m.p. 178–180°, by employing zinc cyanide,[5] which avoids the use of anhydrous hydrogen cyanide. The salt is readily prepared by treating an aqueous solution of sodium cyanide with magnesium chloride, to precipitate the impurities of sodium hydroxide and sodium carbonate, then filtering and precipitating zinc cyanide by adding an equivalent of zinc chloride in alcohol.

2. The oxidation of *o*-orsellinaldehyde is rendered much smoother if the phenolic groups are blocked.

3. The "monocarbomethoxy-*o*-orsellinaldehyde-C^{14}" crystallizes in prisms from ligroin, m.p. 79°.

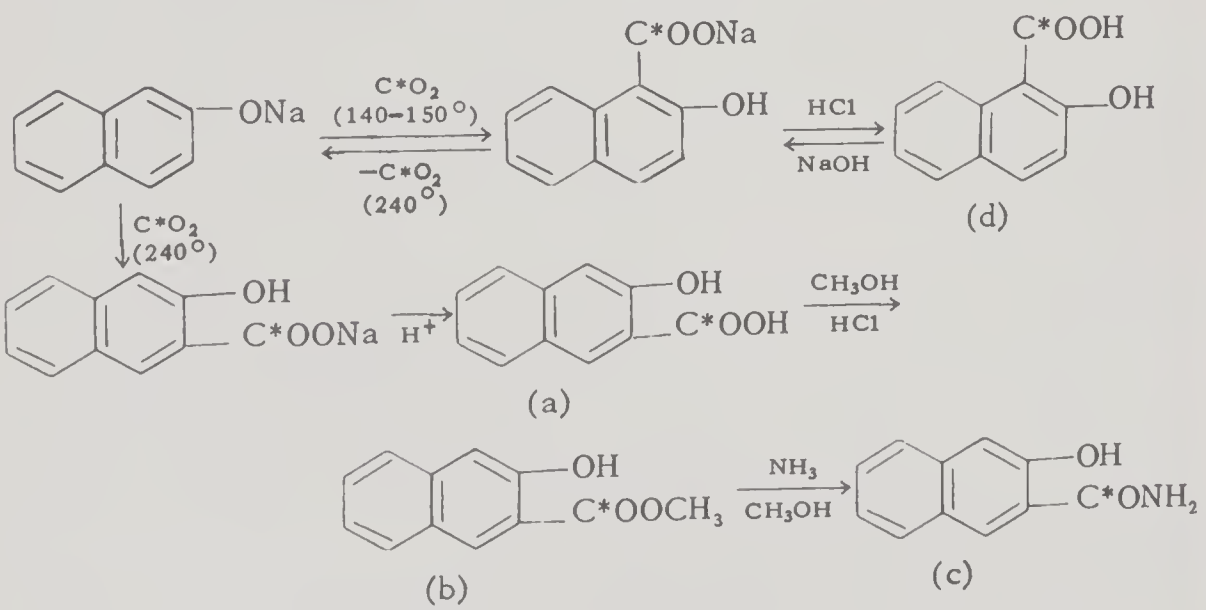
4. The compound develops no color with ferric chloride in alcoholic solution.

5. The compound turns yellow on exposure to light and becomes colorless in the dark.

6. The acid is dehydrated by heating for 2 hours under vacuum over phosphorus pentoxide.

[1] L. Gattermann and M. Köbner, Ber., *32*, 279 (1899).
[2] K. Hoesch, Ber., *46*, 886 (1913).
[3] *Organic Syntheses*, Vol. 23, Wiley, New York, 1943, p. 57.
[4] R. Adams and I. Levine, J. Am. Chem. Soc., *45*, 2373 (1923).
[5] R. T. Arnold and J. Sprung, *ibid*,. *60*, 1699 (1938).

3-HYDROXY-2-NAPHTHOIC-C^{14} ACID



K. Burgdorf, H. Grisebach, H. Kracker and F. Weygand, Ber., 87, 87 (1954).

A. Procedure (Note 1)

(a) *3-Hydroxy-2-naphthoic-C^{14} Acid.* To 1.0 g. of sodium 2-naphthoxide in a 70-ml. bomb tube is added 6.03 mmoles of carbon-C^{14} dioxide by vacuum transfer (liquid nitrogen). The tube is filled with nitrogen, sealed and heated at 240° (Note 2) for 8 hours, then cooled with liquid nitrogen and opened. The unreacted carbon-C^{14} dioxide is recovered in barium hydroxide with use of a nitrogen stream. The reaction residue is extracted with 60 ml. of boiling water (Note 3), and the solution is treated with 0.050 g. of carrier, adjusted at 50° to pH 6.8 with 2 *N* hydrochloric acid and cooled at 5°. The 2-naphthol is removed by centrifuging and washed with water, and the combined solution is acidified to pH 2.8 with 2 *N* hydrochloric acid and refluxed for 1 hour in a stream of nitrogen to decompose the precipitated by-product, 2-hydroxy-1-naphthoic-C^{14} acid (Note 4). The residual solution is neutralized at room temperature (pH 7) with sodium bicarbonate. The generated nonisotopic 2-naphthol is filtered off, and the filtrate is acidified at 80° to pH 2.8 with 2 *N* hydrochloric acid and cooled to 50°. The product is collected, washed with several portions of water and dried at 70°; yield 0.100 g., m.p. 221°. Purification is accomplished by recrystallization from alcohol-water (Note 5).

(b) *Methyl 3-Hydroxy-2-naphthoate-C^{14}.* A stream of hydrogen chloride is passed for 4 hours through a refluxing solution of 1.0 g. of 3-hydroxy-2-naphthoic-C^{14} acid in 10 ml. of methanol (Note 6). Most of the solvent is distilled, and the residue is washed acid-free with water (Note 7).

(c) *3-Hydroxy-2-naphthamide-C^{14}.* A mixture of 1.0 g. of the ester and 10 ml. of methanol, saturated at room temperature with ammonia, is shaken for 12 hours at 40° in a bomb tube. The mixture is evaporated under vacuum, and the pale yellow product is collected and washed with water; yield 89%, m.p. 216° (Note 8).

(d) *2-Hydroxy-1-naphthoic-C^{14} Acid.* To 1.0 g. of potassium 2-naphthoxide in a 90-ml. bomb tube is added 6.03 mmoles of carbon-C^{14} dioxide by vacuum transfer (liquid nitrogen-bath). The tube is filled with nitrogen, sealed and heated at 145–150° for 7 hours, then frozen and opened, the unreacted carbon-C^{14} dioxide being recovered in barium hydroxide solution with use of a nitrogen stream. The residue is dissolved in water at room temperature and treated with 0.100 g. of carrier in 2 *N* sodium hydroxide, then neutralized to pH 6.8 with 2 *N* hydrochloric acid and cooled at 10° to precipitate 2-naphthol, which is filtered off and washed with water. The combined filtrate is acidified to pH 2.8, and the product is collected, washed with water, then diluted with 0.800 g. of carrier and recrystallized from alcohol-water, m.p. 154–155°.

(a′) *3-Hydroxy-2-naphthoic-C^{14} Acid.* A mixture of 1.1 g. of sodium 2-hydroxy-1-naphthoate-C^{14} and nonisotopic carbon dioxide is heated at

240–250° for 6 hours in a 37-ml. bomb tube. The gas is recovered as barium carbonate-C^{14}; molar specific activity 79.5% of that of the 1-naphthoic-C^{14} acid (Note 9). The residue is dissolved in hot water, neutralized to pH 6.8 with 2 *N* hydrochloric acid and cooled at 10°. The 2-naphthol is filtered off, and the filtrate is acidified to pH 2.8 and refluxed for 1 hour to destroy the unreacted starting acid, the evolved carbon-C^{14} dioxide being trapped in barium hydroxide; molar specific activity 73% of that of the starting acid. The 2-naphthol formed is filtered off after the solution is basified at room temperature with sodium bicarbonate. The filtrate is acidified at 70° to pH 2.8 with 2 *N* hydrochloric acid and cooled, and the product is collected, washed with water and recrystallized from alcohol-water; yield 15%, m.p. 221°, molar specific activity 71% of that of the starting acid (Note 10).

B. Notes

1. The preparations are incidental to a tracer study of the mechanism of formation of 3-hydroxy-2-naphthoic acid by carbonation of sodium 2-naphthoxide. The results support the mechanism of Karpukhin[1] as opposed to that of Calcott.[2] Also see Kolbe[3] and Schmitt.[4]

2. The reaction takes a different course at lower temperatures; see (d).

3. A dark insoluble residue is left.

4. The evolved carbon-C^{14} dioxide is collected in barium hydroxide solution.

5. Detailed directions are given by Burgdorf for degrading the acid to carbon-C^{14} dioxide and nonisotopic 2-naphthol with alkaline bisulfite by the method of Bucherer.

6. The ester separates as an oil.

7. An aliquot dissolved in lukewarm sodium acetate solution does not yield a precipitate of the starting acid upon acidification.

8. Detailed directions are given by Burgdorf for the Hofmann degradation of the amide to 3-amino-2-naphthol.

9. The activity agrees with that calculated for uniform exchange between the 1-naphthoic-C^{14} acid and the gas.

10. Degradation by the Bucherer method gives nonisotopic 2-naphthol and barium carbonate-C^{14} having 73% of the molar specific activity of the starting 1-naphthoic acid.

[1]P. P. Karpukhin and J. E. Khusid, Ukrain. Khem. Zhur., *10*, Wiss-tech. Teil, 375 (1935); Chem. Abstracts, *30*, 2191 (1936).

[2]W. S. Calcott, A. R. Hitch and H. W. Mahr, U. S. 1,648,839; Chem. Abstracts, *22*, 432 (1928).

[3]H. Kolbe, Ann., *113*, 125 (1860); J. prakt. Chem., (2) *10*, 98 (1874).

[4]R. Schmitt, J. prakt. Chem., (2) *31*, 397 (1885).

ACID DERIVATIVES

C_1^*-ACETYL HALIDES

METHOD I

$$CH_3C^*OOK \xrightarrow{C_6H_5COBr} CH_3C^*OBr$$

H. S. Anker, J. Biol. Chem., *176*, 1333 (1948).

A. Procedure

Acetyl-1-C^{14} Bromide. To a mixture of 20 mmoles of powdered, anhydrous potassium acetate-1-C^{14} and 20 mmoles of benzoic acid (Note 1) is added 10 ml. of redistilled benzoyl bromide. A plug of glass wool is inserted below the side arm of the distilling flask, and the product is distilled slowly (Note 2); yield 75–90% b.p. 72–76° (Note 3).

Acetyl-1-C^{14} and acetyl-2-C^{13} chlorides are prepared[1] in a similar manner, by refluxing the fused isotopic sodium acetates with a 100% excess of benzoyl chloride. The product is isolated by distillation in 90% yield (Note 4).

METHOD II

$$CH_3C^*OOH \xrightarrow{PBr_3} CH_3C^*OBr$$

M. Calvin and R. M. Lemmon, J. Am. Chem. Soc., *69*, 1232 (1947); M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 210.

A. Procedure

Acetyl-1-C^{14} Bromide. To an excess of stirred phosphorus tribromide (0.35 mole per mole of acid) is added acetic-1-C^{14} acid, slowly from a dropping funnel under anhydrous conditions. The mixture, separating into

a layer of phosphorous acid and an upper layer of acetyl bromide, is heated at 40–50° for 1 hour with stirring. The product is fractionally distilled at 71–76° with the aid of a small fractionating column.

Acetyl-1-C^{14} chloride is prepared similarly,[2] 95%, b.p. 48–54°.

Acetyl-2-C^{14} chloride is prepared in a similar manner, employing phosphorus pentachloride; see acetophenone-2-C^{14}.

METHOD III

J. D. Cox and H. S. Turner, J. Chem. Soc., *1950*, 3176.

A. Procedure (Note 5)

Acetyl-2-C^{14} Chloride. The apparatus consists of a small round-bottomed flask equipped with a nitrogen inlet and a cold finger reflux condenser, and attached through a cold trap to a vacuum manifold (Note 6). To 0.797 g. of frozen acetic-2-C^{14} acid, in an atmosphere of dry nitrogen, is added quickly 9.6 ml. (400% excess) of phthaloyl chloride by pipette (Note 7). The reactants are mixed by gentle shaking as the acid melts, the trap is cooled with Dry Ice, a slow stream of nitrogen is passed through the system, and the mixture is heated at 80°. When acetyl chloride refluxes freely from the cold finger, the inlet tube is rotated to dip below the liquid surface, the gas flow is increased slightly, and the heating-bath is raised to 120°, during 45 minutes, and maintained there for 1 hour. The trap is isolated from the reaction flask, cooled with liquid nitrogen and evacuated to 1 μ. The product is largely freed from hydrogen chloride by cooling at −78° and momentarily opening a stopcock leading to a trap cooled with liquid nitrogen. The product then is vacuum-distilled into a weighing-type trap (Note 8); yield 1.047 g., 101% (Note 9).

B. Notes

1. The benzoic acid was omitted in a similar preparation by Gould,[3] yield 71%.

2. In a preparation[3] from the sodium salt (without benzoic acid) the product distilled when the mixture was heated in an oil-bath at 175°.

3. Heidelberger and Hurlbert[4] have reported a yield of 94%, b. p. 72–80°, by this procedure.

4. The vapor pressure of benzoyl chloride is very low at −5° to −15°.

5. The procedure is an adaptation of the method of Kyrides[5] and is suitable at the 8 to 100-mmole scale.

6. A diagram of the system is presented by Cox.[6]

7. Several preparations of phthaloyl chloride (b.p. 281°) are described by Kyrides.[5]

8. For short periods the product may be stored at −78° in a trap closed by a stopcock, but prolonged storage at room temperature requires sealing *in vacuo* in a break-seal tube.

9. The yield in trial runs was 100–101%. Hydrolysis of an aliquot, followed by titration for chloride and total acid, indicates a composition of 94–95% acetyl chloride, 3–4% hydrogen chloride and 2% acetic acid. The preparation of acetyl-1-C^{14} chloride (93% yield) by this method has been reported by Davis.[7]

C. Other Preparations

The preparation of acetyl-1-C^{14} chloride, essentially by Method I, has been described by Clappison[8] (85%), Strecker[9] (90%) and Bothner-By.[10] Acetyl-1-$C^{13}_{1/1}$-2-$C^{14}_{1/1}$ bromide and acetyl-1-C^{14} bromide have been prepared by Weissbach[11] and Bloch,[12] respectively.

Acetyl-1-C^{14} bromide has been prepared in 72% yield by Schepartz,[13] by a modification of Method II in which the sodium salt was distilled with phosphorus tribromide.

Acetyl-1-C^{13} chloride has been prepared in 80% yield by Olsen,[14] by distilling sodium acetate-1-C^{13} with phosphorus oxychloride. Acetyl-2-C^{14} chloride has been prepared similarly by Ray[15] (70.1% yield, b.p. 50–55 °) from sodium acetate-2-C^{14}; a diagram of the apparatus is given. Acetyl-1-C^{14} chloride has been prepared in like manner by Raaen and Ropp (see methyl 2-thienyl ketone-C^{14}) and by Williams and Ronzio (see acetamide-1-C^{14}).

The preparation of acetyl-1-C^{14} chloride by exchange of nonisotopic acetyl chloride with sodium acetate-1-C^{14} has been described by Curran.[16] From 4 mmoles of acetate and 45 mmoles of acetyl chloride was obtained 26 mmoles of product, b.p. 51–52°, and a fraction presumed to be the crude acetic anhydride, b.p. 125–140°, 0.81 g.

The formation of acetyl-1-C^{14} chloride by an exchange of nonisotopic acetyl chloride with bis(acetic-1-C^{14}) anhydride has been studied by Evans.[17] The reaction has a half-time of about 10 hours at 25°. Separation is effected by distillation with a microfractionating column.

The preparation of acetyl-C^{13}_2 chloride by Method I has been reported by Wood.[18]

[1]M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, pp. 176 and 193.

[2]J. D. Roberts, R. E. McMahon and J. S. Hine, J. Am. Chem. Soc., *72*, 4237 (1950); R. H. Clark and A. Bell, Trans. Roy. Soc. Can., (3) *27*, 97 (1933).

[3]R. G. Gould, A. B. Hastings, C. B. Anfinsen, I. N. Rosenberg, A. K. Solomon and Y. J. Topper, J. Biol. Chem., *177*, 727 (1949).

[4]C. Heidelberger and R. B. Hurlbert, J. Am. Chem. Soc., *72*, 4704 (1950).
[5]L. P. Kyrides, J. Am. Chem. Soc., *59*, 206 (1937).
[6]J. D. Cox, H. S. Turner and R. J. Warne, J. Chem. Soc., *1950*, 3167, Figure 5.
[7]H. W. Davis, E. Grovenstein, Jr., and O. K. Neville, J. Am. Chem. Soc., *75*, 3304 (1953).
[8]J. W. Clappison, J. P. Hummel, W. D. Paul and J. I. Routh, J. Am. Pharm. Assoc., Sci. Ed., *40*, 532 (1951).
[9]H. J. Strecker, H. G. Wood and L. O. Krampitz, J. Biol. Chem., *182*, 525 (1950); M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 176.
[10]A. A. Bothner-By, J. Am. Chem. Soc., *77*, 3293 (1955).
[11]A. Weissbach and D. B. Sprinson, J. Biol. Chem., *203*, 1031 (1953).
[12]K. Bloch, J. Biol. Chem., *179*, 1245 (1949).
[13]B. Schepartz and S. Gurin, J. Biol. Chem., *180*, 663 (1949).
[14]N. S. Olsen, A. Hemingway and A. O. Nier, J. Biol. Chem., *148*, 611 (1943).
[15]F. E. Ray and R. C. Geiser, Cancer Research, *10*, 616 (1950).
[16]G. L. Curran, J. Biol. Chem., *191*, 775 (1951).
[17]E. A. Evans, J. L. Huston and T. H. Norris, J. Am. Chem. Soc., *74*, 4985 (1952).
[18]H. G. Wood, J. Biol. Chem., *194*, 905 (1952).

PROPIONYL-1-C^{14} CHLORIDE

METHOD I

$$CH_3CH_2C^*OOH \xrightarrow{PCl_5} CH_3CH_2C^*OCl$$

J. D. Roberts, R. E. McMahon and J. S. Hine, J. Am. Chem. Soc., *72*, 4237 (1950).

Procedure

Propionic-1-C^{14} acid (15 g.) is converted to the acid chloride by the procedure of Clark and Bell,[1] which follows. The yield is 17.4 g. (93%), b.p. 74–75° (693 mm.).

Propionic acid (20 g., 0.27 mole) is added gradually to 0.27 mole of phosphorus pentachloride in a small distilling flask cooled in an ice-bath. The mixture is fractionally distilled to free it from phosphorus oxychloride; the yield is 77–81%.

METHOD II

$$CH_3CH_2C^*OOH \xrightarrow{C_6H_5COCl} CH_3CH_2C^*OCl$$

J. D. Roberts, R. E. McMahon and J. S. Hine, J. Am. Chem. Soc., *72*, 4237 (1950).

Procedure

Propionic-1-C^{14} acid (20 g.) is converted to the acid chloride by treatment with 63 ml. of benzoyl chloride by the method of Brown,[2] which follows. The yield is 20.3 g. (81%), b.p. 76–77° (740 mm.). The method is general for preparing volatile acid chlorides.

A mixture of 0.25 mole of propionic acid and 0.50 mole of benzoyl chloride (b.p. 194°), in a round-bottomed flask attached to a fractionating column and receiver cooled in a freezing bath, is heated strongly until boiling commences. The product is distilled from the reaction mixture as rapidly as is consistent with good fractionation, usually at a rate such that the temperature at the top of the column does not exceed the boiling point of the acid chloride. The product contains some dissolved hydrogen chloride, but is suitable for most purposes. Purification is effected by refractionation, while maintaining a very high reflux ratio, at first, to dispel the hydrogen chloride, which otherwise carries over considerable product below the true boiling point. The yield of propionyl chloride is 89%, b.p. 77–78.5°.

[1]R. H. Clark and A. Bell, Trans. Roy. Soc. Can., (3) *27*, 97 (1933).
[2]H. C. Brown, J. Am. Chem. Soc., *60*, 1325 (1938).

BENZOYL-C^{14} CHLORIDE

$$C_6H_5C^*OOH \xrightarrow{SOCl_2} C_6H_5C^*OCl$$

C. Heidelberger and H. S. Rieke, Cancer Research, *11*, 640 (1951).

A. Procedure

To a solution of 1.139 g. (9.3 mmoles) of benzoic-C^{14} acid in 10 ml. of dry benzene is added 1.8 ml. (25 mmoles) of purified thionyl chloride (Note 1), and the mixture is refluxed for 3 hours, by which time the evolution of hydrogen chloride has ceased. The solvent is evaporated at reduced pressure, and the last of the unreacted reagent is removed by the vacuum distillation of two added portions of dry benzene. The colorless product is collected by vacuum distillation; yield 1.126 g., 87% (Note 2).

B. Notes

1. Thionyl chloride (50 ml.) may be purified by fractional distillation from 10 ml. of quinoline, in an all-glass apparatus under anhydrous conditions, followed by distillation from 20 ml. of boiled linseed oil.

2. Benzoyl chloride boils at 79° (16 mm.). Ethyl benzoate-C^{14} is prepared[1] in excellent yield by refluxing the acid chloride with an excess of absolute alcohol for 2 hours and fractionally distilling the product.

C. Other Preparations

2,4,6-Trimethylbenzoyl-C^{14} chloride is prepared[2] in the same manner from 2,4,6-trimethylbenzoic-C^{14} acid. The period of refluxing is 2 hours.

The preparation of benzoyl-C^{14} chloride, by essentially this method, has been reported by Dauben[3] (96% yield at the 0.39-mole scale), Stevens,[1] and Schmid[2] (99.5% yield at the 24.5-mmole level).

The preparation of benzoyl-C^{14} chloride by heating benzoic-C^{14} acid with phosphorus pentachloride has been reported by Koton.[4] Reaction with hydrogen peroxide formed benzoyl-C^{14} peroxide.

[1]W. H. Stevens and R. W. Atree, Can. J. Research, *27B*, 807 (1949).

[2]H. Schmid and K. Banholzer, Helv. Chim. Acta, *37*, 1706 (1954); *Organic Syntheses*, Vol. 21, Wiley, New York, 1941, p. 77.

[3]W. G. Dauben, J. C. Reid, P. E. Yankwich and M. Calvin, J. Am. Chem. Soc., *72*, 121 (1950).

[4]M. M. Koton, T. M. Kiseleva and M. I. Bessonov, Doklady Akad. Nauk S.S.S.R., *96*, 85 (1954); Chem. Abstracts, *48*, 1034 (1954).

ACETAMIDE-1-C*

METHOD I

$$CH_3C^*OOH \xrightarrow{(NH_2)_2CO} CH_3C^*ONH_2$$

H. R. V. Arnstein and R. Bentley, J. Chem. Soc., *1951*, 3509.

Procedure

Acetamide-1-C^{13}. Acetic-1-C^{13} acid is converted to the amide by heating with an excess of urea in a stream of nitrogen, according to the method described for the preparation of formamide-C^{13}. Yields[1] are about 95%.

METHOD II

$$CH_3C^*OONa \xrightarrow{POCl_3} \underset{(a)}{CH_3C^*OCl} \xrightarrow{NH_3} \underset{(b)}{CH_3C^*ONH_2}$$

D. L. Williams and A. R. Ronzio, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, unpublished work.

Procedure

(a) *Acetyl-1-C^{14} Chloride.* Sodium acetate-1-C^{14} (0.0816 g., 0.996 mmole) is fused under vacuum in a 25-ml. flask sealed to a 3-inch water-cooled condenser fitted with a connection to a vacuum manifold. The

cooled melt is covered with redistilled phosphorus oxychloride, the condenser is capped, and the mixture is stored at room temperature for 24 hours. The condenser is emptied, the mixture is frozen with liquid nitrogen, and the crude product is distilled (3 hours) at 2 μ from a bath at −10 to −15° into a receiver cooled with liquid nitrogen. The product is redistilled to remove some phosphorus oxychloride.

(b) *Acetamide-1-C*^{14}. To the frozen redistilled acetyl-1-C^{14} chloride is added, by distillation, 2 ml. of anhydrous liquid ammonia. The mixture is melted by replacing the liquid nitrogen-bath with a bath of Dry Ice-acetone. Following the reaction forming acetamide, the temperature is allowed to rise and the excess ammonia is distilled off through a Bunsen valve. The product is separated from the ammonium salts by extraction at room temperature with a total of 40 ml. of chloroform. The solution is evaporated at reduced pressure; yield 0.0472 g. (80.4%), m.p. 81°. The product may be recrystallized from 1 ml. of chloroform at −20° with 88% recovery.

Acetamide-1-C^{14} has been prepared in a similar manner by Anker and Raper;[2] yield 50% (m.p. 78–79°) based on acetyl bromide.

[1]E. Cherbuliez and F. Landolt, Helv. Chim. Acta, *29*, 1438 (1946).
[2]H. S. Anker and R. Raper, J. Biol. Chem., *176*, 1353 (1948).

2-PHENYLACETAMIDE-1-C^{14}

$$C_6H_5CH_2C^*OOH \xrightarrow[HOAc]{NH_4OAc} C_6H_5CH_2C^*ONH_2$$

W. G. Dauben, J. C. Reid, P. E. Yankwich and M. Calvin, J. Am. Chem. Soc., *72*, 121 (1950); *68*, 2117 (1946).

A. Procedure

A mixture of 0.52 g. of phenylacetic-1-C^{14} acid, 0.47 g. of ammonium acetate (Note 1) and 0.78 ml. of glacial acetic acid is distilled very slowly through a modified Claisen flask (Note 2) until the head temperature reached 245°. The residue is dissolved in 1 ml. of boiling water and poured into 2 ml. of 2 *N* sodium hydroxide. The product is collected, washed with two 2-ml. portions of cold water (Note 3) and recrystallized from hot water; yield 40%, m.p. 157–157.5° (Note 4).

B. Notes

1. One set of published directions[1] specifies 0.47 g. of the carbonate, rather than the acetate.

2. The 5-ml. Claisen flask has a 5-cm. indented section serving as a rectifying column, and the upper portion of the flask is wrapped in asbestos. The mixture is heated with an oil-bath, which is brought to 175° over a period of 45 minutes, then gradually raised to 265° during 2 hours.

3. A wash with two 2-ml. portions of ether removes[1] a brown coloration usually found.

4. The yield[1] is 0.25 g. (48%), m.p. 155–158°. The procedure is essentially that of Kao and Ma,[2] which gives an 83% yield of crude amide on the larger laboratory scale. Decreasing the amount of ammonium carbonate[2] leads to a lower yield and partial dehydration of the amide to the nitrile.

C. Other Preparations

2-Phenylacetamide-1-C^{13} has been prepared by Craig,[3] by hydrolysis of phenylacetonitrile-1-C^{13} according to the procedure of Purgotti.[4]

The preparation of 2-phenylacetamide-1-C^{14}, by the Willgerodt reaction from acetophenone-2-C^{14}, has been described by Brown, Cerwonka and Anderson (see 2-phenylacetamide-2-C^{14}).

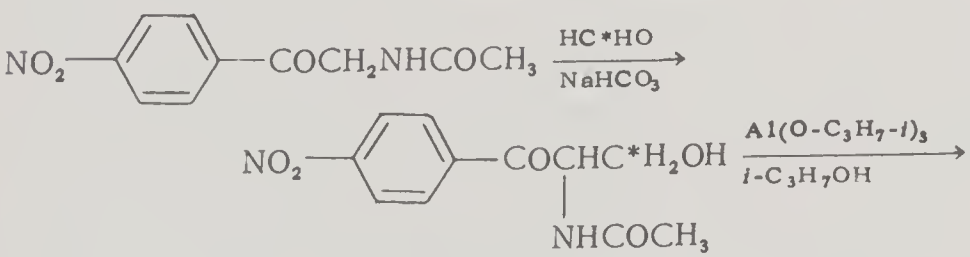
[1]M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon,* Wiley, New York 1949, p. 181.

[2]C. H. Kao and S. Y. Ma, J. Chem. Soc., *1931*, 443.

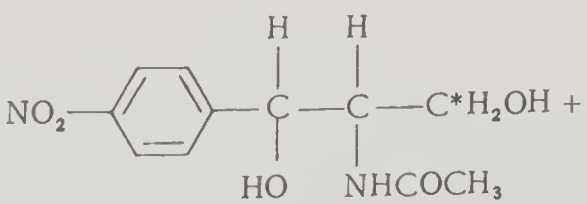
[3]J. T. Craig, J. B. Tindall and M. Senkus, Ann. Chem., Liebigs, *23*, 332 (1951).

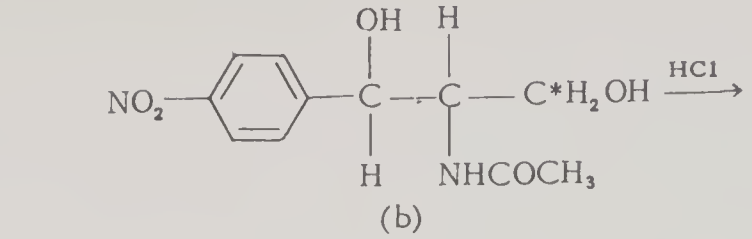
[4]A. Purgotti, Gazz. chim. ital., *20*, 173 (1890); Beilstein, 4th Ed., *9*, 437 (1926).

D-*THREO*-2,2-DICHLORO-*N*-[β-HYDROXY-α-(HYDROXYMETHYL-C^{14})-*p*-NITROPHENETHYL]ACETAMIDE (C_1^{14}-Chloramphenicol)



(a)





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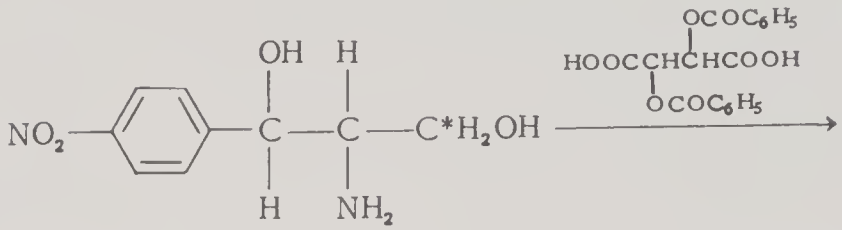
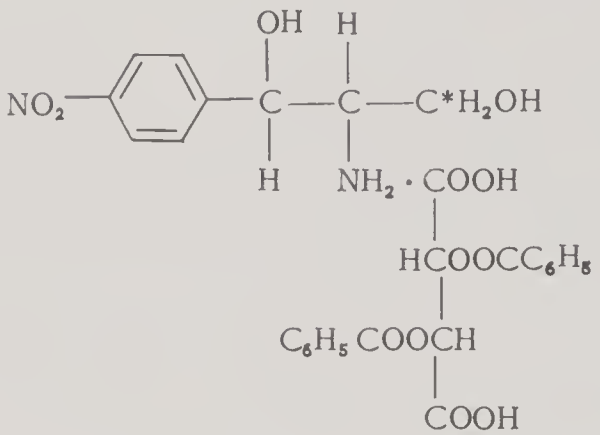
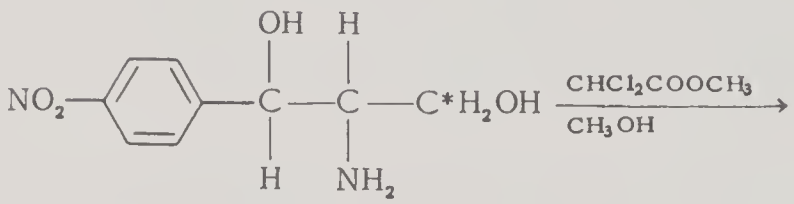
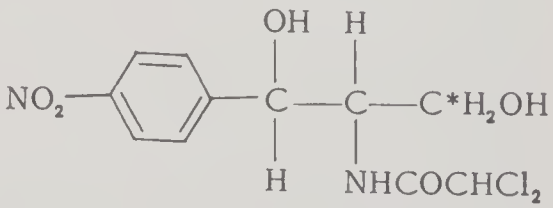


(c)





(d)



(e)

A. Iliceto, Ann. chim. (Rome), *43*, 638 (1953).

A. Procedure (Note 1)

(a) N-[α-(*Hydroxymethyl*-C^{14})-p-*nitrophenacyl*] *acetamide.* A 2.5% solution of 10 mmoles of formaldehyde-C^{14} (Note 2) and 2.22 g. (10 mmoles) of *N*-(*p*-nitrophenacyl)acetamide (Note 3) in alcohol at 35 ± 1° is adjusted to pH 8–8.5 with sodium bicarbonate (Note 4) and stirred for

2 hours. The mixture is cooled, and the product is collected; yield 2.49 g. (85%), m.p. 165.5–167° (dec.).

(b) threo-N-[*β-Hydroxy-α-(hydroxymethyl-C*^{14})-p-*nitrophenethyl*]*acetamide.* The ketone group is reduced by the Meerwein-Ponndorf-Verley method according to the procedure of Long,[1,2] which follows. The ethyl acetate solution is stored at 5° for 4–5 hours to crystallize the product; yield 1.066 g. (42.5%), m.p. 166–166.5° (Note 5).

To a hot stirred solution of 61.2 g. (300 mmoles) of aluminum isopropoxide in 500 ml. of dry isopropyl alcohol in a 1-l. flask equipped with a Hahn condenser[2] is added 53.4 g. (200 mmoles) of *N*-[α-(hydroxymethyl)-*p*-nitrophenacyl]acetamide. Stirring and removal of acetone are continued until the test for acetone is negative (7 hours). The distillation is quite rapid during the last half-hour so that a total of 300 ml. of isopropyl alcohol is removed. The residue is cooled below the boiling point, diluted with 50 ml. of water, refluxed for 15 minutes and filtered. The residue is twice extracted with 250-ml. portions of boiling 80% isopropyl alcohol. The combined extract is evaporated under vacuum, and the residue is refluxed with 100 ml. of ethyl acetate until the oily material dissolves. The solution is cooled to 20°, and the product is collected on a filter, washed with ethyl acetate and dried; yield 25 g., m.p. 159–164°. Purification is accomplished by recrystallization from 80 ml. of boiling water; yield 21.0 g. (41%), m.p. 167–169° (Note 5).

(c) *DL*-threo-*2-Amino-1*-p-*nitrophenyl-1,3-propanediol-3-C*^{14}. The acetamide is hydrolyzed by heating with 5% hydrochloric acid on a steam-bath for 1 hour. The stirred solution is cooled with an ice-bath and made alkaline to thymolphthalein with sodium hydroxide. The product is collected on a filter; yield 0.729 g. (82%), m.p. 137.5–140° (Note 6).

(d) *D*-threo-*2-Amino-1*-p-*nitrophenyl-1,3-propanediol-3-C*^{14}. The resolution of the optical antipodes is accomplished by the procedure of Pratesi[3] (Note 7), which follows; yield: D-form, 0.152 g. (66%), m.p. 162.5–163°; L-form, 0.141 g. (61%), m.p. 163–164.5°.

To a mixture of 8.86 g. of (–)dibenzoyltartaric acid monohydrate and 510 ml. of boiling water is added 5.0 g. of DL-*threo*-2-amino-1-*p*-nitrophenyl-1,3-propanediol. The solution is seeded with D-*threo*-2-amino-1-*p*-nitrophenyl-1,3-propanediol L-dibenzoyltartrate and stored for 24 hours to crystallize. The crude L-*threo*-2-amino-1-*p*-nitrophenyl-1,3-propanediol-[-3-C^{14}] L-dibenzoyltartrate (8.0 g., m.p. 167°) is recrystallized from water; yield 6.63 g., m.p. 174°, $[\alpha]_D^{20}$-85.3° (*c* 1.336, methanol). Evaporation of the mother liquor gives 4.8–5.0 g. of D-*threo*-2-amino-1-*p*-nitrophenyl-1,3-propanediol-[-3-C^{14}] L-dibenzoyltartrate, m.p. 163° from water, $[\alpha]_D^{20}$-62.2° (*c* 1.350, methanol). The salts are decomposed in aqueous suspension by addition of ammonia (1:5), and the pH is adjusted to > 10. The mixture is shaken for 15 minutes and stored for 15

minutes; then the product base is collected and washed with water (Note 8).

(e) *D-threo-2, 2-Dichloro-N-[β-hydroxy-α-(hydroxymethyl-C^{14})-p-nitrophenethyl]acetamide* (Note 9). A mixture of the D-base, 10 volumes of methanol and 1.5 molar equivalents of methyl dichloroacetate is refluxed for 3 hours. The mixture is evaporated, and the residue is recrystallized from 30 parts of boiling water by centrifuging; yield 0.186 g. (78%), m.p. 149.5–150°.

From the L-base is obtained L-*threo*-2,2-dichloro-*N*-[β-hydroxy-α-(hydroxymethyl-C^{14})-*p*-nitrophenethyl]acetamide; yield 0.165 g. (77%), m.p. 149.5–150.5°.

B. Notes

1. The procedure is an adaptation of that of Long.[1]

2. An aqueous solution containing formaldehyde-C^{14} (76–78% yield), sodium chloride and sodium trichloroacetate (prepared by hydrolysis of chloromethyl-C^{14} trichloroacetate at 5–10° with 3 *N* sodium hydroxide) is diluted with alcohol; see formaldehyde-C^{14}, Method V.

3. Directions are given by Long[1] for preparing the starting material in four steps from 4′-nitroacetophenone.

4. The condensation of aromatic ketones with formaldehyde in the presence of more alkaline catalysts (potassium carbonate) leads to the formation of complex products; also see Fuson.[4]

5. From the mother liquor is obtained a small amount of the slower crystallizing *erythro-N*-[β-hydroxy-α-(hydroxymethyl-C^{14})-*p*-nitrophenethyl]acetamide, m.p. 195°.

Purification of the *threo*-form by recrystallization from water (recovery 85%) is generally not necessary.

6. Purification is accomplished by recrystallization from 8 parts of water; recovery 88–90%, m.p. 143–144°.

7. A number of methods of resolution were studied (*q.v.*) to find the one best suited to the semimicro scale.

8. D-*threo*-2-amino-1-*p*-nitrophenyl-1,3-propanediol, m.p. 162–163°, $[\alpha]_D^{20}$ 23.4° (*c* 1.321, methanol); L-*threo*-2-amino-1-*p*-nitrophenyl-1,3-propanediol, m.p. 162–163°, $[\alpha]_D^{20}$ –23.5° (*c* 1.316, methanol).

9. The procedure is an adaptation of that of Alberti,[5] which gave a purer product than did the procedure of Controulis[1] at the small scale.

[1] L. M. Long and H. D. Troutman, J. Am. Chem. Soc., *71*, 2473, 2469 (1949); J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., *idem*, 2463; M. C. Rebstock, H. M. Crooks, Jr., J. Controulis and Q. R. Bartz, *idem*, 2458.

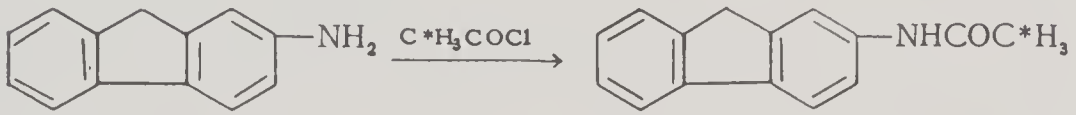
[2] *Organic Reactions*, Vol. II, Wiley, New York, 1944, p. 197.

[3] P. Pratesi, Farmaco, Ed. sci (Pavia), *8*, 41 (1953); Chem. Abstracts, *48*, 635 (1954)

[4]R. C. Fuson, W. E. Ross and C. H. McKeever, J. Am. Chem. Soc., *60*, 2935 (1938).

[5]C. G. Alberti, B. Asero, B. Camerino, R. Sannicolo and A. Vercellone, Chimica e industria (Milan), *31*, 357 (1949); Chem. Abstracts, *46*, 7068 (1952).

N-2-FLUORENYLACETAMIDE-2-C^{14}
(*N*-Acetyl-2-C^{14}-2-aminofluorene)



F. E. Ray and R. C. Geiser, Cancer Research, *10*, 616 (1950).

A. Procedure (Note 1)

To a refluxing mixture of 2.0 g. of dry powdered sodium bicarbonate, 40 ml. of benzene and 4.6 g. of 2-fluorenamine is added the acetyl-2-C^{14} chloride, prepared in 70.1% yield from 3.0 g. of sodium acetate-2-C^{14}. After heating for 30 minutes, the mixture is cooled with ice, and the precipitate is collected and dried. A small amount of precipitate is recovered by diluting the filtrate with ligroin (Note 2). The combined solid is washed with hot water (Note 3) and recrystallized from aqueous ethanol (Note 4). The product is mixed with 0.3 g. of carrier *N*-2-fluorenylacetamide and recrystallized from dilute ethanol; yield 2.3 g. (41.1%), m.p. 194° (Note 5).

B. Notes

1. The original paper contains a diagram of the apparatus used.

2. The solid is principally 2-fluorenamine hydrochloride, m.p. 216–220°.

3. The crude product melts at 150–165°.

4. The product, melting at 172–178° and unchanged by recrystallization, is believed to be an equilibrium mixture of *N*-2-fluorenylacetamide and 2-fluorenamine.

5. Since the low yield is due to repeated recrystallization, the addition of carrier prior to crystallization is recommended.

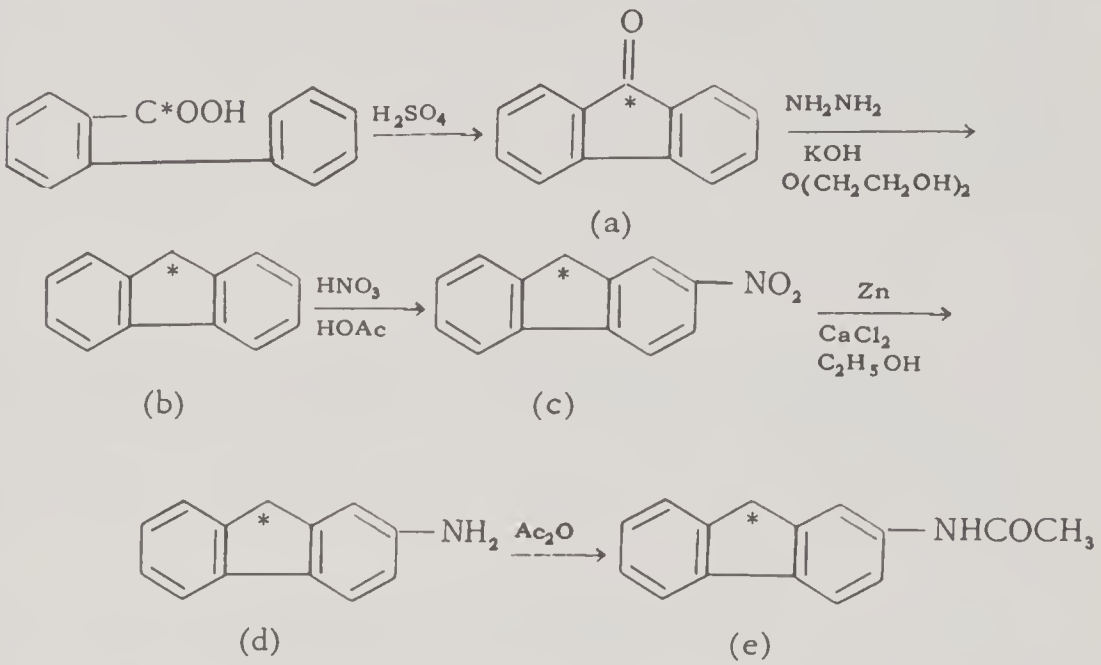
C. Other Preparations

In a similar manner is prepared[1] *N*-2-fluorenyl-9-C^{14}-benzamide, (*N*-benzoyl-2-aminofluorene-9-C^{14}). To a mixture of 0.100 g. of 2-fluorenamine-9-C^{14} in 2 ml. of hot pyridine is added 0.080 g. of benzoyl chloride. The mixture is heated at 100° for 5 minutes, then cooled, diluted with an equal volume of water, and acidified with concentrated hydrochloric acid.

The product is collected, washed with water, and twice recrystallized from dilute alcohol; yield 0.120 g. (86%), m.p. 219–220.°

[1]H. R. Gutmann and J. H. Peters, Cancer Research, *13*, 415 (1953).

N-2-FLUORENYL-9-C^{14}-ACETAMIDE
(*N*-Acetyl-2-aminofluorene-9-C^{14})



C. Heidelberger and H. S. Rieke, Cancer Research, *11*, 640 (1951).

A. Procedure

(a) *9-Fluorenone-9-C^{14}* (Note 1). To a solution of 2.2 ml. of sulfuric acid and 0.4 ml. of water is added 0.794 g. of 2-biphenylcarboxylic-C^{14} acid. The mixture is heated at 85° for 30 minutes, then poured into a mixture of ice and water, and the crude product is filtered off; yield 0.713 g., 99% (Note 2).

(b) *Fluorene-9-C^{14}* (Note 1). A mixture of 0.713 g. of 9-fluorenone-9-C^{14}, 0.51 g. of potassium hydroxide, 5 ml. of diethylene glycol and 0.7 ml. of 85% hydrazine hydrate is refluxed for 3 hours. The nearly colorless solution is cooled and poured into water, and the product is filtered off; yield 0.598 g., 92% (Note 3).

(c) *2-Nitrofluorene-9-C^{14}*. The fluorene-9-C^{14} is nitrated in glacial acetic acid solution by the method of Kuhn,[1] and the product is collected by filtration in a Gooch crucible; yield 0.523 g. (71%), m.p. 156–158° (Note 4).

(d) *2-Fluorenamine-9-C^{14}, (2-Aminofluorene-9-C^{14})*. The 2-nitrofluorene-9-C^{14} is reduced in 94% yield (Note 5) by the procedure of Kuhn,[1] which follows.

A mixture of 46.7 ml. of ethanol, 10.3 ml. of water, 0.6 g. of calcium chloride, 1.7 g. of 2-nitrofluorene and 5.7 g. of zinc dust is refluxed for 5 hours, then filtered, diluted with 114 ml. of water and stored overnight. The flocculent precipitate is collected and dried.

(e) *N-2-Fluorenyl-9-C^{14}-acetamide.* The acetylation of 0.355 g. of 2-fluorenamine-9-C^{14} is carried out in 97% yield (Note 6), by the procedure of Ray and Geiser,[2] which follows.

To a boiling solution of 1.3 g. of 2-fluorenamine-9-C^{14} in 7.5 ml. of benzene is added slowly 0.82 g. of acetic anhydride. The precipitate is collected and dissolved in 12 ml. of boiling ethanol, and the solution is treated with water to the point of incipient turbidity, then set aside to crystallize.

B. Notes

1. The procedure is a modification of the isotopic syntheses described by Ray and Geiser.[2]

2. There is no residual carboxylic acid.[2] The yield is 88%, if concentrated[2] sulfuric acid is employed. Harris[3] has reported a 95% yield, m.p. 79–80°, employing concentrated acid.

3. The product melts[2] at 109–111°. Harris[3] has reported a 97% yield, m.p. 112–114°, obtained by the use of ethylene glycol and no alkaline catalyst. Alkali promotes the formation of 9,9′-bifluorene.

4. A yield of 79%, m.p. 155–156°, by this procedure has been reported.[2]

5. The yield of colorless product is 0.410 g., m.p. 125–127°, after recrystallization from water. By the same procedure Ray[2] has obtained the product in 90% yield, while Gutmann and Peters[4,5] have reported a yield of 63% (m.p. 126°) based on fluorene. Radiochemical purity (m.p. 127–128° cor.) was shown[5] by a single radioactive peak (R_f 0.89) on scanning of paper chromatograms[6] developed by ascending chromatography in 1-butanol saturated with 3 *N* ammonium hydroxide. Paper electrophoresis[7] in 20% acetic acid gave[5] a single radioactive peak (mobility 4.5×10^{-5} cm./sec./volt/cm.).

6. The yield of recrystallized product is 0.403 g., m.p. 193–194°. Ray[2] has obtained a yield of 84%, m.p. 194–195°, while Gutmann and Peters reported a melting point of 197–198°. An extremely sensitive test for the presence of diazotizable amino groups is described by Gutmann.[8] The compound is shown to be isotopically pure[4,5] by paper electrophoresis[7] and by paper chromatography, developed with butanol saturated with 3 *N* ammonium hydroxide (R_f 0.90). Chromatography with the solvent system hexane-benzene (2:1) saturated with water also is described.[5]

C. Other Preparations

The preparation of fluorene-9-C^{14} by the Clemmensen reduction of 9-fluorenone-9-C^{14} has been reported by Ray and Geiser[2] (70% yield, m.p. 109–111°) and by Gutmann and Peters[4] (m.p. 110°, yield 90%; or 84.5% based on 2-biphenylcarboxylic-C^{14} acid). The latter investigators modified the procedure by introducing glacial acetic acid (3 ml.) to aid in the solution of the fluorene-9-C^{14} (2.5 mmoles). Short reaction time with 7% hydrochloric acid gives the intermediate 9-fluorenol (m.p. 144–145°), while longer reduction gives fluorene (80% yield) and 9,9′-bifluorene (20% yield, m.p. 245–246°).[3]

[1]*Organic Syntheses*. Coll. Vol. II, Wiley, New York, 1943, p. 447.

[2]F. E. Ray and R. C. Geiser, Cancer Research, *10*, 616 (1950); Science, *109*, 200 (1949).

[3]A. S. Harris, E. N. White and D. McNeil, J. Chem. Soc., *1955*, 4216.

[4]H. R. Gutmann and J. H. Peters, Cancer Research, *13*, 415 (1953); J. Biol. Chem., *211*, 63 (1954).

[5]J. H. Peters and H. R. Gutmann, J. Biol. Chem., *216*, 713 (1955).

[6]H. L. Demorest and R. Baskin, Anal. Chem., *26*, 1531 (1954).

[7]J. H. Peters and H. R. Gutmann, J. Am. Chem. Soc., *76*, 2267 (1954).

[8]H. R. Gutmann, G. E. Kiely and M. Klein, Cancer Research, *12*, 350 (1952).

2-PHENYLACETANILIDE-1-C^{14}

$$\text{I. } C_6H_5CH_2C^*OOH \xrightarrow{C_6H_5NCO} CO_2 + C_6H_5CH_2C^*ONHC_6H_5$$

$$\text{II. } 2\,C_6H_5CH_2C^*OOH \xrightarrow{2\,C_6H_5NCO} CO_2 + (C_6H_5CH_2C^*O)_2O + (C_6H_5NH)_2CO \xrightarrow{\Delta} CO_2 + 2\,C_6H_5CH_2C^*ONHC_6H_5$$

A. Fry, J. Am. Chem. Soc., *75*, 2686 (1953).

A. Procedure (Note 1)

The apparatus consists of a reaction vessel, a trap at −80° and a spiral bubbler filled with 1 *N* carbonate-free sodium hydroxide. Phenyl isocyanate and phenylacetic-1-C^{14} acid (10 mmoles each) are washed into the reaction vessel with 5 ml. of xylene. The system is swept with a slow stream of dry carbon dioxide-free air until no further carbon dioxide is evolved (Note 2). The mixture is filtered, and the residue is extracted with ether and with hot benzene to remove bis(phenylacetic-1-C^{14}) anhy-

dride, phenyl isocyanate and the product (Note 3). The combined filtrate is washed with 10% sodium carbonate solution, dried and evaporated. The crude product is recrystallized from alcohol; m.p. 117–118°. The molar specific activity is the same as that of the starting material.

B. Notes

1. This procedure is incidental to a study of the mechanism of reaction between isocyanates and carboxylic acids.[1] The carbon dioxide liberated comes from the isocyanate.

2. The reaction time is established by trial runs. The carbon dioxide is precipitated as barium carbonate and weighed; yield 6–8 mmoles. The yield becomes quantitative (see the second reaction) when the reaction temperature is raised. A reaction between bis(acetic-1-C^{14}) anhydride and 1,3-diphenylurea at 135° yielded acetanilide-1-C^{14} having the same molar specific activity as the starting material.

3. The residue consists of nonisotopic 1,3-diphenylurea (carbanilide), m.p. 237.5–238°.

C. Other Preparations

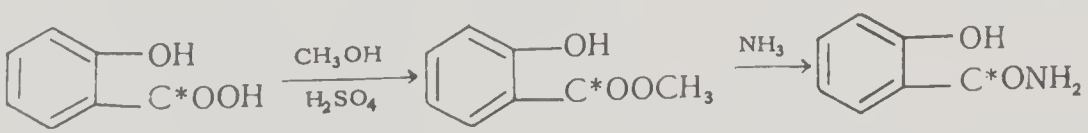
A similar preparation with *p*-bromophenyl isocyanate yields 4′-bromo-2-phenylacetanilide-1-C^{14}, m.p. 174–175°, needles from benzene-cyclohexane, or ether. The carbon dioxide contains about 1% of the initial activity, possibly due to a very small amount of acylative decarboxylation of the phenylacetic-1-C^{14} acid, such as occurs in the Dakin-West reaction.[2]

In a similar manner from acetic-1-C^{14} acid and phenyl isocyanate or *p*-bromophenyl isocyanate are prepared acetanilide-1-C^{14} (m.p. 113–114°) and 4′-bromoacetanilide-1-C^{14} (m.p. 164–165°), respectively.

[1] C. Naegeli and A. Tyabji, Helv. chim. Acta, *17*, 931 (1934); *18*, 142 (1935); W. Dieckmann and F. Breest, Ber., *39*, 3052 (1906).

[2] H. Dakin and R. West, J. Biol. Chem., *78*, 91 (1928).

SALICYLAMIDE-C^{14}



(a) (b)

H. G. Mandel and C. L. Brown,[1] Department of Pharmacology, the George Washington University School of Medicine, Washington, D. C., private communication.

A. Procedure (Note 1)

(a) *Methyl Salicylate-C^{14}*. A mixture of 3.0 g. (22 mmoles) of salicylic-C^{14} acid, 12 ml. of anhydrous methanol and 5 drops of concentrated sulfuric acid is refluxed for 24 hours under anhydrous conditions.

(b) *Salicylamide-C^{14}*. The crude ester mixture is cooled to 0° and treated with 20 ml. of concentrated ammonium hydroxide. After occasional shaking during a period of 6 days, the two layers become a homogenous solution. The mixture is stored for 7 additional days in a refrigerator, then cooled in an ice-bath and acidified with hydrochloric acid. The precipitate is collected on a filter, and the unreacted salicylic acid is separated by solution in saturated sodium bicarbonate solution (Note 2). The insoluble product is collected and recrystallized from water; yield 1.8 g. (60%), m.p. 139–140°. The yield is nearly quantitative based on unrecovered salicylic acid. Chromatography in a system of benzene-glacial acetic acid-water (40 : 40 : 20, v/v) using a descending paper strip and radio-assaying, or developing the color with diazotized *p*-nitroaniline (Note 3), shows the presence of only one component (Note 4). The molar specific activity agrees with that of the starting material.

B. Notes

1. The conversion of salicylic acid into the amide through the acid chloride is not satisfactory. The procedure is based on the report of Kline.[2]

2. Salicylic-C^{14} acid (1.0 g.) is recovered by acidification of the alkaline solution and extraction with ethylene chloride.

3. The method of paper chromatography is that developed for phenolic compounds by Bray;[3] another is described by Weygand.[4]

4. $R_f = 0.83$ with Schleicher and Schüll 589 green label paper, and 0.66 with Whatman No. 3 paper. As further proof of purity, a countercurrent distribution in a system of 0.1 *M* citrate buffer (pH 5.0) and isoamyl alcohol, when measured spectrophotometrically, agrees with the theoretical values calculated for the pure compound by the method of Way and Bennett;[5] the partition ratio is 0.046.

Purification may be accomplished[4] by vacuum sublimation (120° bath temperature, 5 μ).

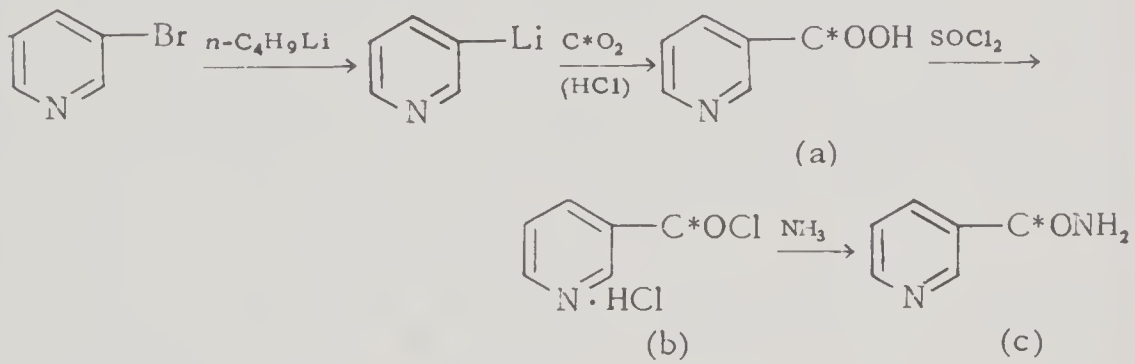
C. Other Preparations

A similar preparation of methyl salicylate-C^{14} (HCl catalyst) and the conversion to salicylamide-C^{14} have been described in detail by Weygand;[4] yield 83% based on salicylic acid, m.p. 138°.

[1]H. G. Mandel, V. W. Rodwell and P. K. Smith, J. Pharmacol. Exp. Therap., *106*, 433 (1952).

[2]E. T. Kline, J. Chem. Ed., *19*, 332 (1942).
[3]H. G. Bray, W. V. Thorpe and K. White, Biochem. J., *46*, 271 (1950).
[4]F. Weygand, A. Becher, D. Feldmann and O. A. Grosskinsky, Hoppe-Seyler's Z. physiol. Chem., *292*, 138 (1953).
[5]E. L. Way and B. M. Bennett, J. Biol. Chem., *192*, 335 (1951).

NICOTINAMIDE-C^{14}



A. Murray III, W. W. Foreman and W. Langham, J. Am. Chem. Soc., *70*, 1037, (1948); A. Murray, III and A. R. Ronzio, Atomic Energy Commission Report, AECU-87; Nuc. Sci. Abstracts, *2*, 910 (1949).

A. Procedure

(a) *Nicotinic-C^{14} Acid* (Note 1). The apparatus is a modification of that described for the preparation of *p*-aminobenzoic-C^{14} acid, by a similar halogen-metal exchange reaction. The lower addition tube is fitted with a jacket containing a Dry-Ice-cooling mixture, and the siphon is eliminated from the reaction flask, as, in this case, the intermediate organolithium compound does not precipitate from solution.

To a stirred solution of 1.63 mmoles of butyllithium (Note 2) in 2.76 ml. of ether, at -35° under nitrogen, is added, over a 4-minute period, 2.44 mmoles of redistilled 3-bromopyridine in 5 ml. of absolute ether maintained at -35°. The cooling bath is exchanged for one at -78° and, after 1 minute of stirring, the mixture is carbonated with the carbon-C^{14} dioxide previously liberated from 0.1608 g. (0.814 mmole) of barium carbonate-C^{14}. The mixture is hydrolyzed with 3 ml. of 2.5 *N* nitric acid (Note 3) and continuously extracted with ether for 3-4 hours. The aqueous solution is made alkaline and extracted for 4-6 hours; then the aqueous solution is adjusted to pH 3, and the product is isolated by ether extraction for 48-72 hours. Solvent evaporation and desiccation over phosphorus pentoxide give the crude acid; yield 62.4% based on barium

carbonate, or 70.3% based on unrecovered carbon-C^{14} dioxide (Note 4). Purification is accomplished by vacuum sublimation (100–130° at 0.1 μ) and recrystallization from methanol (–80°) or absolute ethanol; yield 0.030 g., m.p. 225–228° (uncor.) (Note 5).

(b) *Nicotinoyl-C^{14} Chloride Hydrochloride* (Note 6). A stirred mixture of 0.100 g. of dry, crude nicotinic-C^{14} acid and 5.0 ml. of purified thionyl chloride (see benzoyl-C^{14} chloride) is refluxed for 75 minutes in an all-glass system, protected from moisture by a tower filled with calcium chloride. The excess thionyl chloride is removed through the vertical condenser by evaporation at the reduced pressure of a water pump (Note 7).

(c) *Nicotinamide-C^{14}*. (Note 6). To the acid chloride hydrochloride is added approximately 15 ml. of anhydrous liquid ammonia by distillation, condensing with a Dry Ice-bath. The mixture is stirred (magnetic stirring bar) at room temperature, and the excess ammonia is evaporated overnight through a Bunsen valve. The flask is evacuated briefly to remove the remaining ammonia; then the residue is dissolved in a few ml. of water. The yellow solution is filtered, strongly acidified with hydrochloric acid and continuously extracted with ether for 2 hours. The aqueous solution is made basic with sodium bicarbonate and extracted continuously with pure ethyl acetate for 18 hours. The extract is treated with charcoal, concentrated by evaporation in an air steam and dried in a desiccator; crude yield 0.097 g. (98%). Purification is accomplished by dissolution in 15 ml. of boiling toluene (Note 8) and fractional recrystallization at 0°; yield 0.069 g. of colorless needles (69.6%), m.p. 125–127° (Note 9).

B. Notes

1. The procedure is adapted from the method of Gilman and Spatz.[1]
2. See *p*-aminobenzoic-C^{14} acid, Note 4.
3. The recovery of unreacted carbon-C^{14} dioxide is 11.4%.
4. The combined crude yield from two isotopic runs at the 2.8-mmole scale was 84.5% based on barium carbonate, while the figure at the 1.32-mmole scale was 79.9%.
5. The yield of crude nicotinic-C^{13} acid, prepared in two batches by this procedure, is 81.9% based on 21 mmoles of barium carbonate-C^{13} taken. Purification through the insoluble silver salt, followed by ether extraction, gives the acid in 55.4% yield, m.p. 227–229°. A determination of nicotinic acid is outlined by Goldblith,[5] and the effect of irradiation by cathode rays is described. The paper chromatography and radioautography of nicotinic-C^{14} acid and nicotinamide-C^{14} are described by Johnson.[1a]

6. The procedure is a modification of that described by Späth and Spitzer.[2]

7. The hydrochloride, m.p. 155.5–156.5°, sublimes[2] at 170–190°.

8. A colored impurity, which becomes more apparent as the scale of the reaction is lowered, is filtered off.

9. The yield of an isotopic run at the 2.6-mmole scale was 77.4%; yields in trial runs have been as high as 94%, m.p. 124.5–127°. Purification may be accomplished by vacuum sublimation.

C. Other Preparations

Nicotinic-C^{14} acid has been prepared by Beher,[3] by essentially the described procedure. The yield at the 73.5-mmole scale was 65%, m.p. 236–237° (cor.).

The preparation of 3,4,5-trimethoxybenzoic-C^{14} acid by the carbonation of 3,4,5-trimethoxyphenyllithium at –60° has been described in detail by Numerof;[4] yield 90% at the 0.15-mmole level.

The preparation of benzamide-C^{14} by amination of benzoyl-C^{14} chloride has been reported by Clark;[6] radiochemical yield 76.2% based on carbon dioxide, m.p. 121.5°.

The preparation of xanthene-9-carboxylic-C^{14} acid has been described in detail by Nakai[7] with the aid of a diagram of the apparatus adapted to hand shaking. The halogen-metal exchange reaction was carried out under reflux for 30 minutes with 2.0 g. of xanthene and 12.5 mmoles of butyllithium. The carbonation (5.25 mmoles of carbon-C^{14} dioxide) was effected at the temperature of a salt-ice bath and completed with non-isotopic carbon dioxide (1 hour). The acid-insoluble product was collected, washed with water and reprecipitated from alkali; yield 1.354 g., g., m.p. 215–216°, radiochemical yield 50.5%.

[1]H. Gilman and S. M. Spatz, J. Am. Chem. Soc., *62*, 446 (1940).

[1a]B. C. Johnson and P-H. Lin, J. Am. Chem. Soc., *75*, 2971 (1953); P-H. Lin and B. C. Johnson, *idem*, 2974.

[2]E. Späth and H. Spitzer, Ber., *59*, 1477 (1926).

[3]W. T. Beher, W. M. Holliday and O. H. Gaebler, J. Biol. Chem., *198*, 573, (1952).

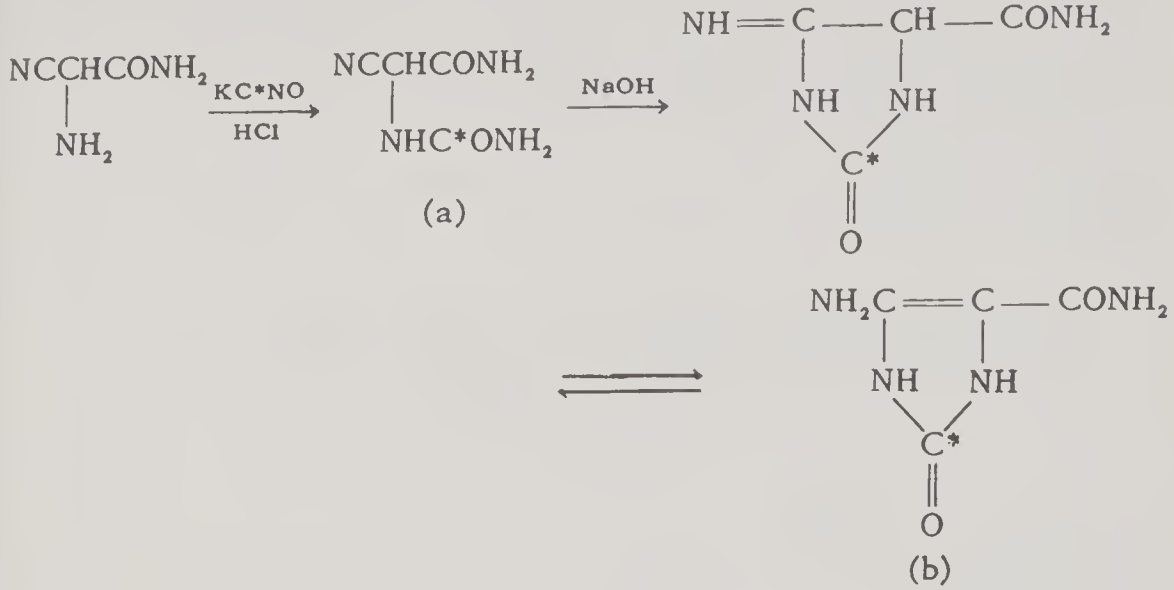
[4]P. Numerof, M. Gordon and J. M. Kelly, J. Pharm. Exp. Therap., *115*, 427 (1955); M. Kohn and S. Grün, Monatsh., *46*, 86 (1925).

[5]S. A. Goldblith, B. E. Proctor, J. R. Hogness and W. H. Langham, J. Biol. Chem., *179*, 1163 (1949).

[6]M. T. Clark, E. G. Hendley and O. K. Neville, J. Am. Chem. Soc., *77*, 3280 (1955).

[7]R. Nakai, M. Sugii and H. Tomono, Bull. Inst.,Chem. Research, Kyoto Univ., 33, 211 (1955); R. R. Burtner and J. W. Cusic, J. Am. Chem. Soc., *65*, 1582 (1943).

5-AMINO-2-OXO-4-IMIDAZOLINE-4-CARBOXAMIDE-2-C^{13}



L. H. Smith, Jr. and P. Yates, J. Am. Chem. Soc., 76, 6080 (1954).

A. Procedure

(a) *2-Ureido-C^{13}-2-cyanoacetamide.* To a stirred solution of 20 g. of 2-amino-2-cyanoacetamide (Note 1) in 30 ml. of 6 *N* hydrochloric acid at 5° is added, during 30 minutes, 125 ml. of aqueous potassium cyanate-C^{13} solution prepared from 8.44 g. of potassium cyanide-C^{13} (Note 2). The mixture is stirred for 15 minutes and stored at 5° overnight, and the product is collected; yield 12.7 g. (69% based on cyanide) (Note 3).

(b) *5-Amino-2-oxo-4-imidazoline-4-carboxamide-2-C^{13}.* To a solution of 1.0 g. of 2-ureido-C^{13}-2-cyanoacetamide and 10 ml. of water at 85° is added 1.2 ml. of 5% sodium hydroxide. The solution is heated for 10 minutes, cooled slowly and stored overnight at 5°. The product is collected; yield 0.75 g. (75%) (Note 4).

B. Notes

1. Directions are given by Smith for preparing the starting material by reduction of cyanohydroxyiminoacetamide [see 4(or 5)-amino-5(or 4)-imidazolecarboxamide-4(or 5)-C^{14} hydrochloride, Method II] with aluminum amalgam.[1]

2. The cyanate-C^{14} solution prepared by Method I is concentrated at 40° and 20 mm.

3. The product may be recrystallized from water, forming prisms from concentrated solution (m.p. 202–203° dec.) and needles from dilute solution (m.p. 200–201° dec.).

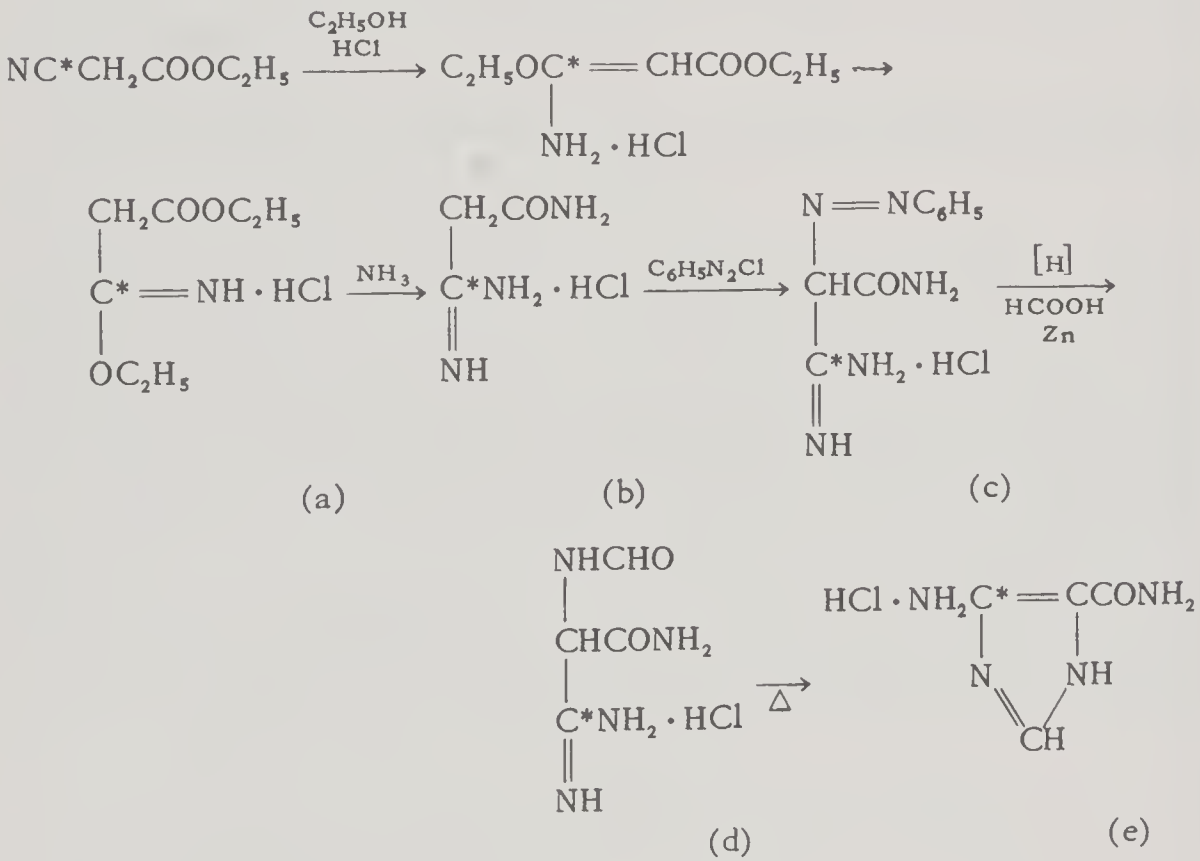
4. The product, twice recrystallized from water, is completely soluble in cold, dilute hydrochloric acid; m.p. above 370°, darkening above

200°; the ultraviolet spectrum is given. 2,4-Dioxo-5-imidazole-carboxamide-2-C^{13} is formed by hydrolysis with one equivalent of dilute hydrochloric acid; m.p. 254–255° (dec.) from dilute alcohol. Hydrolysis with excess hot concentrated hydrochloric acid forms hydantoin-2-C^{13}, m.p. 222–224° from ethanol-ether.

[1]V. Cerchez and Dumitresco-Calesiu, Bull. soc. chim. France, (5) *1*, 852 (1934).

4(or 5)-AMINO-5(or 4)-IMIDAZOLECARBOXAMIDE-4(or 5)-C^{14} HYDROCHLORIDE

METHOD I



H. G. Mandel and C. L. Brown, Department of Pharmacology, The George Washington University School of Medicine, Washington, D. C., private communication; G. M. Conzelman, Jr., H. G. Mandel and P. K. Smith, J. Biol. Chem., *201*, 329 (1953).

A. Procedure (Note 1)

(a) *Ethyl 2-Carbethoxyacetimidate-1-C^{14} Hydrochloride* (Note 2). Anhydrous hydrogen chloride is bubbled through a solution, cooled in an ice-salt bath, of 5.1 g. (45 mmoles) of ethyl cyano-C^{14}-acetate, 3.1 ml. (50 mmoles) of absolute ethanol and 4 ml. of absolute ether, until the gain

in weight is 2.4 g. The mixture is seeded and stored in a refrigerator for 72 hours with gradual addition of 100 ml. of ether in occasional small portions, until crystallization is complete. The colorless product is collected on a filter and dried in a vacuum desiccator; yield 8.2 g. (92%), m.p. 100–101°.

(b) *2-Amidino-C^{14}-acetamide Hydrochloride.* Dry ammonia is bubbled for 1 hour through a suspension of the ethyl imino ether hydrochloride in 80 ml. of absolute ethanol, previously saturated with anhydrous ammonia. The mixture is stored at room temperature for 24 hours, with occasional shaking until solution is effected. The mixture is seeded and stored for 24 hours; then the precipitated product is collected and dried in a desiccator; yield 5.1 g. (88%), m.p. 177–178° (Note 3).

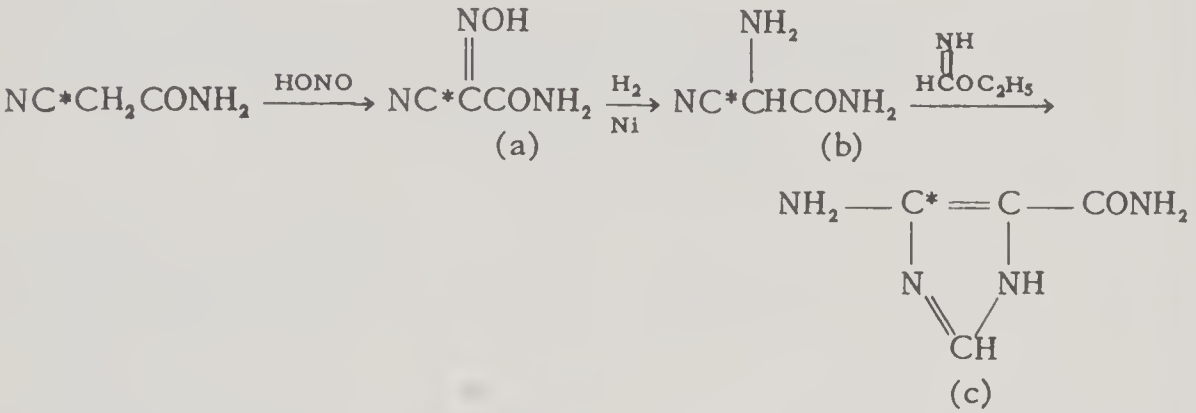
(c) *2-Amidino-C^{14}-2-phenylazoacetamide Hydrochloride.* A diazonium solution is prepared by treating a solution of 4.0 ml. of aniline in 25 ml. of 6 *N* hydrochloric acid at 0° with 3.0 g. of sodium nitrite in 20 ml. of water. The excess nitrous acid is decomposed with urea in 5 minutes, and the solution then is added to an aqueous solution of the amidine hydrochloride. The mixture is treated with 100 ml. of saturated sodium acetate solution and stored in a refrigerator for 3 hours. The yellow precipitate is collected and dried in a desiccator; yield 8.4 g. (95%), m.p. 198–200° (Note 4).

(d) *2-Amidino-C^{14}-2-formamidoacetamide Hydrochloride.* A solution of the amidinophenylazoacetamide in 50 ml. of 98% formic acid is treated with 10 g. of zinc dust. The mixture is warmed at 70° for 15 minutes and, after discharge of the azo color, the precipitate is separated by centrifuging and washed with several portions of formic acid. The combined filtrate and washings are evaporated at 60° under reduced pressure; water is added, and the mixture is evaporated to dryness at a bath temperature of less than 45° (Note 5). The residue is dissolved in water, and the solution is saturated with hydrogen sulfide. The precipitate is separated by centrifuging, and the supernatant solution is evaporated under vacuum at 45°, with several additions of absolute alcohol to remove water from the syrup. The residue is dissolved in a small amount of methanol and treated with an excess of methanol saturated with hydrogen chloride, followed by a large volume of ether to precipitate the product. After 30 minutes, the precipitated oil is separated from the supernatant solution of formanilide, washed with absolute ether, and crystallized in the refrigerator. The product is washed with a small amount of acetone and vacuum-dried (Note 6).

(e) *4(or 5)-Amino-5(or 4)-imidazolecarboxamide-4(or 5)-C^{14} Hydrochloride.* The 2-amidino-C^{14}-2-formamidoacetamide hydrochloride is heated at 175° at reduced pressure. The solid melts and resolidifies in about 5 minutes as the ring closure is effected. The product is recrystallized from a minimal volume of aqueous solution by the addition of absolute

ethanol and ether, collected, and dried in a desiccator (Note 7); yield 3.9 g. (m.p. 258–260° dec.), 70% based on the azo derivative, or 50% based on ethyl cyanoacetate. A paper strip chromatogram on Whatman No. 1 paper, developed in a system of butanol-diethylene glycol-water (4:1:1 v/v), shows only one radioactive peak, R_f 0.63 (Note 8).

METHOD II



C. S. Miller, S. Gurin and D. W. Wilson, J. Am. Chem. Soc., *74*, 2892 (1952); Science, *112*, 654 (1950).

A. Procedure

(a) *2-Cyano-C^{14}-2-hydroximinoacetamide.* A solution of 2-cyano-C^{14}-acetamide is nitrosated, in aqueous acetic acid at 10–15°, by treatment with a 20% excess of sodium nitrite. The mixture is stirred in the cold for 6 hours and stored overnight in a refrigerator. The mixture cautiously is decomposed with cold concentrated hydrochloric acid, then concentrated at 50–60° under reduced pressure to half-volume (Note 9) and cooled overnight. The crystalline product is collected and separated from sodium chloride by dissolution in absolute alcohol, filtration and evaporation of the solvent; yield 77%, m.p. 181° (dec.) (Note 10).

(b) *2-Amino-2-cyano-C^{14}-acetamide.* The 2-cyano-C^{14}-hydroxyiminoacetamide (4 g.) is reduced by hydrogenation at 3 atmospheres over 4 g. of aged Raney nickel in a suspension of 16 g. of anhydrous sodium sulfate and absolute methanol. After the calculated hydrogen uptake, the solution is filtered and used directly (Note 11).

(c) *4(or 5)-Amino-5(or 4)-imidazolecarboxamide-4(or 5)-C^{14} Hydrochloride* (Note 12). A 20% excess of ethyl formimidate is added to the cold methanol solution of the unstable 2-amino-2-cyano-C^{14}-acetamide (Note 13). The product is isolated and purified through the picrate, m.p. 239–240° (dec.) (cor.) when immersed in a bath preheated to 227°. The hydrochloride is obtained by saturating a mixture of the picrate and anhydrous acetone with hydrogen chloride, while the temperature is

maintained constant; yield 25–30% based on 2-amino-2-cyanoacetamide, m.p. 262.5–263.5° (cor.) (dec.) when immersed in a bath preheated to 245°.

B. Notes

1. The method is a modification of that reported by Shaw and Wooley.[1]

2. The procedure is a modification of the method of Glickman and Cope.[2]

3. The compound may be recrystallized by dissolution in a minimal volume of water, followed by dilution with five volumes of absolute ethanol and sufficient absolute ether to induce crystallization.

4. An additional crop of product is obtained when the mother liquor is treated with 200 ml. of the sodium acetate and stored in the refrigerator for 1 week. The brown-colored precipitate is purified by extraction with a 50% mixture of acetone and ether, the insoluble solid being collected. Also see Smith.[3]

5. It is important to keep the temperature low during this evaporation.

6. The acetone precipitates additional product and extracts some of the by-products. Shaw and Woolley[1] have reported a 63% yield of the hemihydrate, m.p. 93–95°, which is unstable to recrystallization; an analytical sample melted at 95–96°.

7. The somewhat gray product contains a small amount of the dihydrochloride. The picrate melts at 238–239° and the free base melts at 168–169°.

8. The spots may be developed by diazotization.[4] Purity was also indicated by an 8-plate countercurrent distribution in a system of 1*M* phosphate buffer at pH 6.5 and butanol (partition ratio 1.82), when read spectrophotometrically. The ultraviolet absorption spectrum agrees with values reported in the literature.[4] Shaw[1] has reported an over-all yield of 30% based on ethyl cyanoacetate.

9. The oxides of nitrogen are removed at the water-pump

10. The pure compound melts at 182–183° (dec.), after recrystallization from water.

11. The unstable product in a trial run was acetylated with acetic anhydride and characterized as 2-acetamido-2-cyanoacetamide, m.p. 173.5–174.5°.

12. A similar preparation of the imidazole has been reported[5] in which 2-amino-2-cyanoacetamide is condensed with formamidine hydrochloride in boiling methanol.

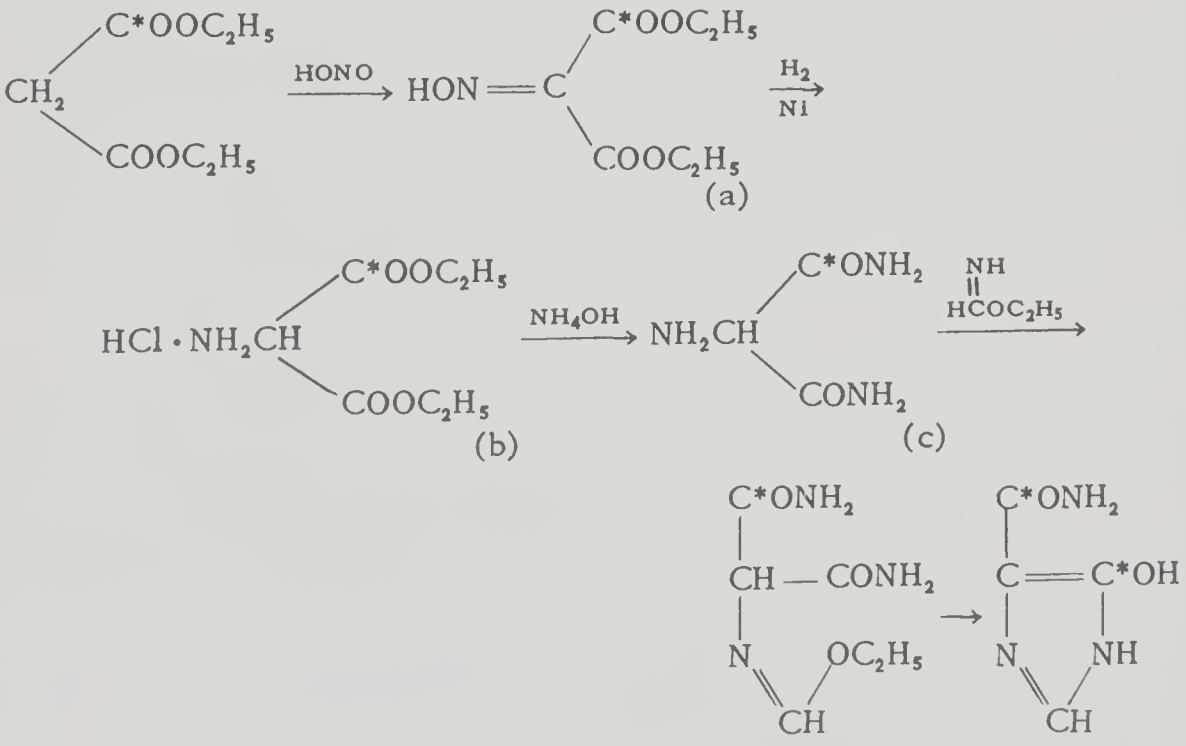
13. Directions are given for preparing both imino esters, ethyl formimidate and ethyl acetimidate. 4(or 5)-Amino-2-methyl-5(or 4)-imidazolecarboxamide is prepared in 37% yield from the latter compound and isolated as the picrate, m.p. 240° (dec.)

C. Other Preparations

4-Amino-5-imidazolecarboxamide-4-C^{13} has been prepared[6] by the method of Shaw and Wooley;[1] yield 48% based on potassium cyanide. Purification was accomplished by crystallization from aqueous solution as the picrate, m.p. 238–240° (dec.), and conversion to the hydrochloride by Method II, the last traces of picric acid being removed by continuous extraction with ethereal hydrogen chloride. A solution in 0.01 *M* phosphate buffer (pH 7.0) shows maximal absorption at 267 mμ, E11,200 (authentic purified sample, E11,300). The intermediates are 2-amidino-C^{13}-acetamide hydrochloride, 2-amidino-C^{13}-2-phenylazoacetamide hydrochloride and 2-amidino-C^{13}-2-formamidoacetamide hydrochloride. In order to prepare the azo compound as a yellow crystalline product it was necessary to maintain the pH at 4 by repeated additions of sodium acetate solution.

[1]E. Shaw and D. W. Wooley, J. Biol. Chem., *181*, 89 (1949).
[2]S. A. Glickman and A. C. Cope, J. Am. Chem. Soc., *67*, 1017 (1945).
[3]L. H. Smith, Jr. and P. Yates, J. Am. Chem. Soc., *76*, 6080 (1954).
[4]J. S. Gots, Arch. Biochem., *29*, 222 (1950).
[5]A. H. Cook, I. Heilbron and E. Smith, J. Chem. Soc., *1949*, 1440.
[6]J. E. Seegmiller, L. Laster and D. Stetten, Jr., J. Biol. Chem., *216*, 653 (1955).

4(or 5)-HYDROXY-5(or 4)-IMIDAZOLECARBOXAMIDE-C^{14}_{1}-4(or 5)C^{14}_{1}



C. S. Miller, S. Gurin and D. W. Wilson, J. Am. Chem. Soc., *74*, 2892 (1952).

A. Procedure (Note 1)

(a) *Ethyl Hydroxyiminomalonate-1-C^{14}.* Ethyl malonate-1-C^{14} is nitrosated by the procedure of Galat,[1] which follows.

To a stirred mixture of 0.2 mole of ethyl malonate and 0.6 mole of glacial acetic acid, maintained below 20°, is added a solution of 0.55 mole of sodium nitrite in 55 ml. of water. The mixture is stirred for 4.5 hours at room temperature, then extracted with chloroform, and the solvent is distilled under reduced pressure on a water-bath; the yield of residual product is 38 g. (Note 2).

(b) *Ethyl Aminomalonate-1-C^{14} Hydrochloride.* The ethyl hydroxyimino ester is reduced by the method of Levene and Schormuller,[2] which follows

A mixture of 25 g. of purified ethyl hydroxyiminomalonate, 45 g. of absolute alcohol and 5 g. of Raney nickel catalyst is shaken with hydrogen at 40 p.s.i. until absorption ceases (4-5 hours). The catalyst is removed by centrifuging, and the solvent is evaporated under reduced pressure. The syrup is taken up in 250 ml. of absolute ether, then saturated with dry hydrogen chloride and stored in a refrigerator for 2 hours. The product is collected on a filter and washed with ether; yield 18–21 g. (Note 3).

(c) *2-Aminomalonamide-1-C^{14}.* Ethyl aminomalonate-1-C^{14} hydrochloride is treated with cold aqueous ammonium hydroxide solution.[3] The product is collected and washed with water and alcohol; the yield is nearly quantitative (Note 4).

(d) *4(or 5)-Hydroxy-5(or 4)-imidazolecarboxamide-$C^{14}_{1/1}$-4(or 5)-$C^{14}_{1/1}$* (Note 5). To a cold, stirred aqueous solution of 6.79 g. of 2-aminomalonamide-1-C^{14} containing one equivalent of hydrochloric acid is added a 70% molar excess of ethyl formimidate, and the mixture is stored overnight at 5° (Note 6). The product is filtered off, washed with a minimal volume of water, decolorized with charcoal in hot aqueous solution (Note 7) and crystallized. After several recrystallizations, the yield is 1.97 g. (30%), m.p. above 250° (dec.) (Note 8).

B. Notes

1. The method is based upon a method reported by Finger.[4]

2. Purified ethyl hydroxyiminomalonate, b.p. 145–150° (3 mm.), has been prepared in 87% yield, by Redemann and Dunn,[5] with the use of sodium ethoxide and butyl nitrite.

3. A sample recrystallized from alcohol-ether melts at 170°. The preparation of the compound as the free base (65% yield) is described by Redemann and Dunn.[5]

4. After recrystallization from hot water or dilute alcohol, the melting point is 192° with decomposition beginning at 185–186°.

5. The method is a modification of a method by Schmidt.[6] The ethyl formimidate is prepared in 50–85% yield by rigorous drying of an ether solution of the ethyl formimidate hydrochloride with potassium carbonate, evaporation of the solvent, and vacuum distillation of the product. The straw-colored liquid freezes at about −18°, in which condition it is best stored. The ethyl formimidate hydrochloride is prepared[6] by mixing stoichiometric amounts of anhydrous ethanol, hydrogen cyanide and hydrogen chloride in an inert solvent (petroleum ether) with cooling.

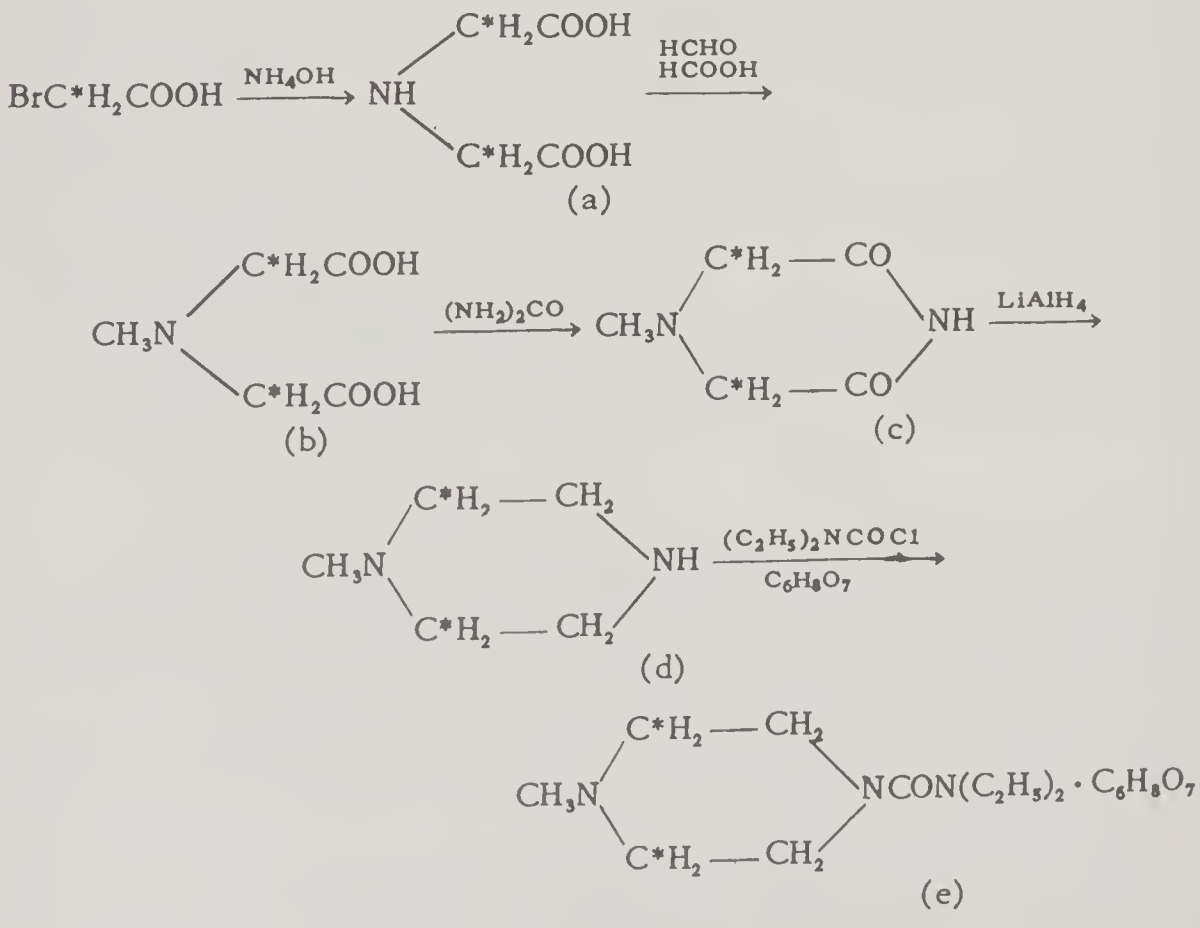
6. The ethyl ester, m.p. 229–230°, is formed in poor yield when equal amounts of ethyl aminomalonate and ethyl formimidate are set aside for three days in dioxane at 5°.

7. Unboiled water produces a small amount of green pigment.

8. The 2-methyl homologue (m.p. above 250° dec.) is prepared in 70% yield from ethyl acetimidate, by the same procedure.

[1]A. Galat, J. Am. Chem. Soc., *69*, 965 (1947).
[2]P. A. Levene and A. Schormuller, J. Biol. Chem., *106*, 595 (1934).
[3]O. Piloty and J. Neresheimer, Ber., *39*, 514 (1906).
[4]H. Finger, J. prakt Chem., *76*, 93 (1907); Chem. Abstracts, *1*, 123 (1908).
[5]C. E. Redemann and M. S. Dunn, J. Biol. Chem., *130*, 341 (1939).
[6]E. Schmidt, Ber., *47*, 2545 (1914).

N,*N*-DIETHYL-4-METHYL-1-PIPERAZINECARBOXAMIDE-3,5-C_2^{14} DIHYDROGEN CITRATE



B. H. Chase and A. M. Downes, J. Chem. Soc., *1953*, 3874.

A. Procedure

(a) *Iminodiacetic-2,2′-C_2^{14} Acid* (Note 1). A concentrated aqueous solution of the residues from two glycine-2-C^{14} preparations,[1] after removal of ammonia and the bulk of the product, is separated by partition chromatography on a column of Dowex-50 (Note 2) which is developed with 1.5 *N* hydrochloric acid. The first 850 ml. of eluate is discarded; then 10.6-ml. fractions are collected automatically. Periodic sample aliquots are evaporated to dryness and assayed for activity, which is plotted against tube number. The aliquots of eluate contributing to each peak of activity are combined, evaporated to dryness at 50° under reduced pressure and identified by paper chromatography (Note 3). A clear solution of 0.2040 g. of iminodiacetic-2,2′-C_2^{14} acid hydrochloride, in 1.5 ml. of water, diluted with 10 ml. of absolute ethanol, is treated dropwise with pyridine until precipitation is complete; then the mixture is stored at 0° for 2 hours to crystallize; yield 0.1123 g. (Note 4). The radiochemical yield may be raised to 93% by addition of 73 mg. of carrier iminodiacetic acid (in two portions) to the mother liquor, followed by concentration and precipitation with absolute ethanol; the additional crops are 0.0659 g. and 0.0296 g.

(b) *N-Methyliminodiacetic-2,2′-C_2^{14} Acid.* A mixture of 1.33 g. of iminodiacetic-2,2′-C_2^{14} acid, 1.5 g. of 95% formic acid and 1.2 g. of 40% formaldehyde is refluxed for 7 hours. The solvent is evaporated under diminished pressure, and the residue is triturated with 15 ml. of absolute alcohol; yield 1.39 g. (95%), m.p. 215–216° (dec.) (Note 5). Paper chromatography and radioautography show the presence of only one compound.

(c) *1-Methyl-3,5-piperazinedione-2,6-C_2^{14}.* A mixture of 0.1464 g. of *N*-methyliminodiacetic-2,2′-C_2^{14} acid and 0.062 g. of urea is heated at 160° in the open tube of a small sublimation apparatus. When frothing subsides, the bath temperature is raised to 170° during one hour. The product is sublimed at 170–190° (15 mm.) and crystallized from ethanol; yield 0.0768 g., 60% (Note 6).

(d) *1-Methylpiperazine-2,6-C_2^{14} Hydrochloride.* To a stirred solution of 0.230 g. of lithium aluminum hydride in 25 ml. of absolute ether is added a solution of 0.1728 g. of 1-methyl-3,5-piperazinedione-2,6-C_2^{14} in 5 ml. of dry tetrahydrofuran. The mixture is refluxed for 4 hours, cooled and hydrolyzed by the dropwise addition of 5 ml. of water. The mixture is acidified with 2 *N* hydrochloric acid, stirred for one hour and concentrated by distillation. The residue is made basic with sodium hydroxide solution, and the crude product is steam-distilled into 10 mmoles of dilute hydrochloric acid. The distillate (1.25 l.) is evaporated to dryness under diminished pressure, the residue is crystallized from absolute ethanol containing a few drops of concentrated hydrochloric acid, and the product is vacuum-dried; yield 0.1883 g., 72% (Note 7).

(e) N,N-*Diethyl-4-methyl-1-piperazinecarboxamide-3,5-*C_2^{14} *Dihydrogen Citrate.* To a mixture of 0.0976 g. of 1-methylpiperazine-2,6-C_2^{14} dihydrochloride monohydrate and 0.22 ml. of triethylamine in 4 ml. of anhydrous chloroform is added a solution of 0.0765 g. of diethylcarbamoyl chloride (Note 8) in 1 ml. of chloroform, and the mixture is stored overnight at room temperature. The mixture is evaporated in a stream of air, and the residue is mixed with 15 ml. of absolute ether; and then the triethylamine hydrochloride is filtered off and washed with ether. The combined solution is concentrated to 5 ml. and treated with a solution of 0.115 g. of citric acid in 10 ml. of ether. The crystalline product is collected, washed with ether and dried under vacuum; yield 0.1799 g. (90%). The molar specific activity is the same as that of the starting material. Paper chromatography and radioautography show the presence of only one compound, and purity is further confirmed by isotopic dilution analysis.

B. Notes

1. The classical method of preparing glycine, by ammonolysis of chloroacetic acid with ammonium hydroxide,[2] results in the formation of considerable amounts of by-product iminodiacetic acid as well as nitrilotriacetic acid and glycolic acid. Robertson[3] has studied extensively the velocity and course of the reaction, and determined that the by-products are formed in appreciable amounts if the concentration of glycine is allowed to exceed 1 mole per cent of that of the ammonia. Cheronis and Spitzmueller[4] have found that the presence of ammonium carbonate (lower pH) and formation of an unstable amino acid carbamate inhibit the formation of the secondary and tertiary-amino compounds.

2. The column (3.5 × 90-cm.) is prepared from 1 kg. of 200-400-mesh resin, which is washed with 4 *N* hydrochloric acid and water.

3. A spot is neutralized with ammonia, and the chromatogram is developed with phenol-water-ammonia. Identity may be confirmed by isotopic dilution analysis.

4. Directions are given by Chase for the preparation of iminodiacetic acid, m.p. 235° (dec.) (88% yield), by hydrogenolysis[5] of *N*-benzyliminodiacetic acid over palladium. The latter compound, m.p. 204°, is prepared in 50% yield from benzylamine and chloroacetic acid, by a modification of the method of Berchet.[6]

5. The yield on the 0.1444-g. scale is 92%. *N*-Methyliminodiacetic acid may be prepared in 63–71% yield from chloroacetic acid and methylamine by the method of Berchet.[6]

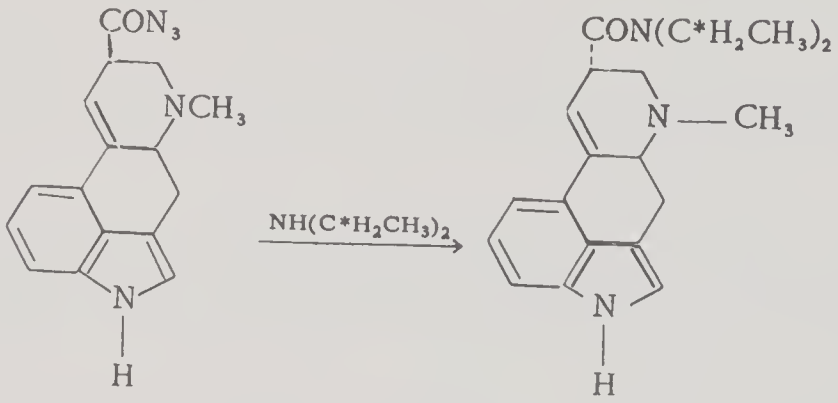
6. The yield on the 3-g. scale is 87%, m.p. 98–100.5°; recrystallization from propanol raises the melting point to 103–104°. The use of urea in preparing imides[7] is rather novel.

7. The salt is a dihydrochloride monohydrate, m.p. 84–86°; a sample dried under vacuum over phosphorus pentoxide at 100° melts at 242–243°. The yield is 77% on a 20-g. scale. The dipicrate melts at 265° (dec.)

8. This preparation is by a modification of the method of Kushner.[8] Diethylcarbamoyl chloride, b.p. 190–195°, may be obtained by distillation of diethyloxamic acid with phosphorus pentachloride, or by reacting diethylamine in benzene solution with a toluene solution of phosgene, by the procedure of Lumiere.[9]

[1]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 300.
[2]K. Kraut, Ann., *266*, 292 (1891).
[3]G. R. Robertson, J. Am. Chem. Soc., *49*, 2889 (1927).
[4]N. D. Cheronis and K. H. Spitzmueller, J. Org. Chem., *6*, 349 (1941).
[5]L. Birkofer, Ber., *75*, 429 (1942).
[6]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 397.
[7]J. Guareschi, Bull. soc. chim., *49*, 299 (1888).
[8]S. Kushner, L. M. Brancone, R. I. Hewitt, W. L. McEwen, Y. SubbaRow, H. H. Stewart, R. J. Turner and J. J. Denton, J. Org. Chem., *13*, 144 (1948).
[9]A. L. Lumiere and F. Perrin, Bull. soc. chim., (3) *31*, 689 (1904).

N,*N*-BIS(ETHYL-1-C^{14})LYSERGAMIDE (C_1^{14}-LSD 25)



A. Stoll, J. Rutschmann and A. Hofmann, Helv. Chim. Acta, *37*, 820 (1954).

A. Procedure (Note 1)

A solution of 0.150 g. of diethylamine-1,1′-C_2^{14} hydrochloride in 0.5 ml. of water is frozen on the walls of a tube immersed in a Dry Ice-acetone bath. The tube is filled with pellets of potassium hydroxide and attached, by way of a tube packed with soda lime, to a reaction tube containing a mixture of 0.400 g. of crystalline D-isolysergoyl azide (Note 2) and 4 ml. of dioxane frozen with a bath of liquid nitrogen, Figure III, 1. The amine is liberated by thawing of the frozen hydro-

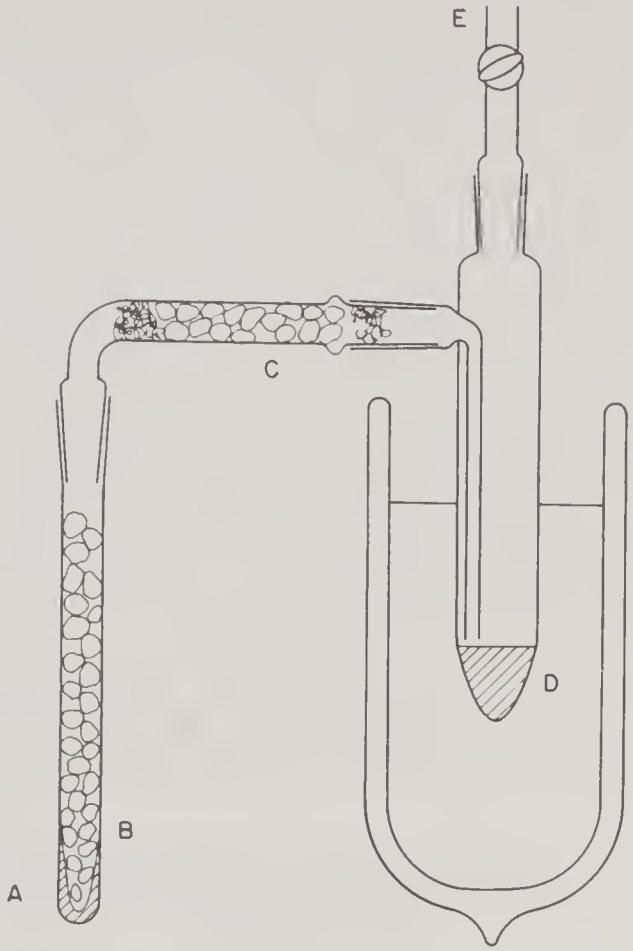


Fig. III, 1. Apparatus for condensation of diethylamine with isolysergoyl azide (A. Stoll, J. Rutschmann and A. Hofmann). A, frozen solution of amine hydrochloride; B, potassium hydroxide; C, soda lime; D, frozen azide solution; E, outlet to vacuum manifold.

chloride solution and reaction with the alkali. The mixture is frozen with liquid nitrogen, the apparatus is evacuated to 1 μ, and the diethylamine-1,1′-C_2^{14} is distilled into the reaction tube (final heating, 40° for 15 minutes). The system is filled with dry nitrogen, and the closed reaction tube is heated at 55° for 2 hours. The mixture is evaporated under vacuum, and the residue is dissolved in dilute tartaric acid solution, which then is basified with sodium bicarbonate and extracted with a total of 100 ml. of ether. The crude product obtained by evaporation is dissolved in chloroform and chromatographed on 40 g. of aluminum oxide. The first fraction of eluate, which exhibits an intense blue fluorescence under ultraviolet light, is evaporated to dryness. Dissolution in a few drops of ethyl acetate and dilution with 20 volumes of ether give a first crop of pure product which is collected on Hyflo filter aid; yield 0.060 g., m.p. 82–83° (dec.).

The combined residue, obtained from the mother liquors and further fractions of eluate, is dissolved in 5 ml. of absolute alcohol and treated with 0.5 ml. of 4 *N* potassium hydroxide (Note 3). The solution is stored at room temperature for 1.5 hours, then acidified with dilute tartaric acid

solution, concentrated and extracted with ether after being basified with sodium bicarbonate. The extract is evaporated, and the residue is purified by chromatography; yield 0.060 g. From the mother liquor is obtained a final crop (0.020 g.), making the total yield 0.140 g. The molar specific activity is the same as that of the starting material.

B. Notes

1. The procedure is an adaptation of that of Stoll.[1] Because of the sensitivity of the product to light and oxygen, it is recommended that the preparation be shielded from daylight and completed as quickly as possible (30 hours).

2. The crystalline starting material is freshly prepared by concentrating an ethereal extract from a solution of D-isolysergoyl azide hydrochloride and sodium bicarbonate. The preparation of the salt by the action of nitrous acid on the hydrazide is described by Hofmann.[2]

3. The *N,N*-bis(ethyl-1-C^{14})isolysergamide is rearranged in alkaline solution. *N,N*-Diethyl-D-isolysergamide melts at 182° (dec.).[1] Lysergic and isolysergic acids differ in the configuration of the carboxyl group and hydrogen at C-8.[3]

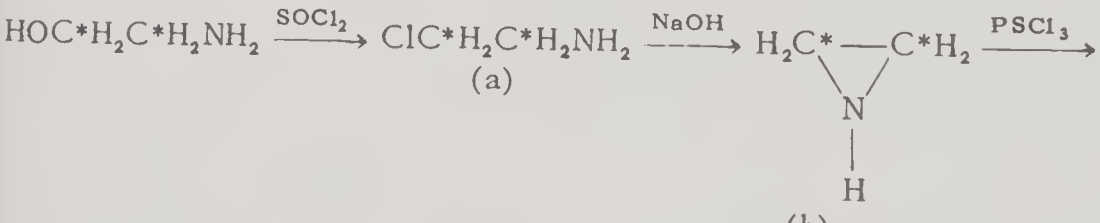
[1] A. Stoll and A. Hofmann, Helv. Chim. Acta, *26*, 944 (1943).

[2] A. Hofmann, Helv. Chim. Acta, *30*, 44 (1947).

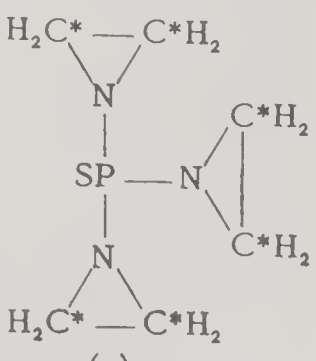
[3] A. Stoll, J. Rutschmann, A. Hofmann and H. H. Günthard, Helv. Chim. Acta, *37*, 2039 (1954).

1,1′,1″-PHOSPHINOTHIOYLIDYNETRISAZIRIDINE-C_6^{14}

(*N,N′,N″*-Triethylenethiophosphoramide-C_6^{14})



(a) (b)



(c)

D. L. Williams, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, unpublished work.

A. Procedure

(a) *2-Chloroethylamine-C_2^{14} Hydrochloride.* A suspension of 1.0153 g. (10.4 mmoles) of 2-aminoethanol-C_2^{14} hydrochloride (Note 1) in 10 ml. of 1,1,1-trichloroethane is cooled in an ice-bath. A solution of 3 ml. of thionyl chloride in 7 ml. of 1,1,1-trichloroethane is added, and the mixture, protected from moisture by a calcium chloride tube, is slowly warmed and then refluxed on a steam-bath for 12 hours. After 4 hours of heating and also after 9 hours, 1-ml. amounts of thionyl chloride are used to wash down the condenser (Note 2). The excess thionyl chloride is destroyed with the calculated amount of methanol. Then 10 ml. of 1,1,1-trichloroethane is added, and the volume of solvent is reduced to 8–10 ml. by distillation. The mixture is cooled in an ice-bath, and the solvent is removed with a filter stick (Note 3). The nearly colorless crystalline product is washed with 3 portions of ether and dried under vacuum. The yield of 2-chloroethylamine-C_2^{14} hydrochloride is 1.1390 g. (0.00982 mole, 94.4%) (Note 4).

(b) *Aziridine-C_2^{14}, (Ethylenimine-C_2^{14}).* The above 2-chloroethylamine-C_2^{14} hydrochloride is dissolved in methanol and transferred to a 25-ml. reaction flask equipped with a sealed-on 6-inch water condenser attached to a semiball joint. The reaction flask is attached to a vacuum manifold, and the solvent is distilled *in vacuo*, first from a bath at -20° and finally at room temperature. With the residue cooled in a bath at -10° and water at $10–15^\circ$ circulating through the condenser, 7.6 ml. of 4 *N* sodium hydroxide is added to the hydrochloride (Note 5). The apparatus is reattached to the manifold, and the mixture is heated in a water-bath at 50° for 1 hour. The cooling water is removed from the condenser completely with a stream of air, and the reaction flask, including the condenser, is immersed in liquid nitrogen. The product and most of the water from the reaction mixture are distilled *in vacuo* into a 200-ml. flask containing 40 g. of sodium hydroxide pellets (Note 6). As the flask warms, the water and the aziridine-C_2^{14} distill onto the cold mass of sodium hydroxide. Then after 4 hours, the aziridine-C^{14} and a small amount of water are distilled *in vacuo* into a second flask containing 5 g. of freshly ignited calcium oxide. After 2 hours of contact with the calcium oxide, the dry product is vacuum-distilled into an evacuated weighing flask. The yield of aziridine-C_2^{14} is 0.3539 g. (83.4%).

(c) *1,1',1"-Phosphinothioylidynetrisaziridine-C_6^{14}, (N,N',N"-Triethylenethiophosphoramide-C_6^{14})* (Note 7). The 0.3539 g. of aziridine-C_6^{14} is distilled *in vacuo* into a 100-ml. 2-necked flask containing a solution of 1.49 ml. (28.66 mmoles) of ethylenimine and 5.2 ml. (36.9 mmoles) of triethylamine in 20 ml. of benzene. The contents of the flask are melted, mixed and partially refrozen. Then the flask is filled with dry nitrogen and is quickly removed from the manifold and equipped with a mercury-

sealed stirrer and a compensated dropping funnel. With the stirred solution cooled in a bath at 0°, a solution of 2.09 g. (12.3 mmoles) of thiophosphoryl chloride in 10 ml. of benzene is added dropwise during 30 minutes. Stirring is continued for 30 minutes as the solution temperature rises to room temperature. The reaction mixture is diluted with 30 ml. of ether and is then filtered through a medium siltered glass funnel. The filtrate is evaporated to dryness under vacuum. The residue is extracted repeatedly with ether (Note 8). The ether solution is filtered, and the combined filtrate is evaporated to dryness under low vacuum. The crude crystalline product is colorless and weighs 1.9704 g. (84.7%). The crude product is dissolved in 45 ml. of warm hexane, and the clear solution is filtered (Note 9). The filtrate is cooled overnight at 0° and for an additional 2 hours at −40°. The mother liquor is removed with a filter stick, and the crystalline product is dried under vacuum; the yield is 1.2884 g. (55%). The product is dissolved in 50 ml. of warm petroleum ether and filtered from a small amount of oily residue. The solution is cooled in a bath at −50° for 1 hour, and the solvent is then removed with a filter stick. After drying under vacuum, the yield of crystalline product, m.p. 53–54°, is 1.2272 g. (52.7%) (Note 10).

B. Notes

1. Acetylene-C_2^{14},[1] ethylene-C_2^{14} oxide[2] and 2-aminoethanol-C_2^{14} [3] were prepared according to procedures already described. The latter compound was isolated by extraction with chloroform from an aqueous solution saturated with potassium carbonate.

2. As the reaction nears completion, the oily mixture of starting material and product begins to crystallize and finally breaks up into fine particles.

3. Most of the brown-colored material formed during the reaction is dissolved in the solvent.

4. In a preliminary experiment on the 10-mmole scale, the yield of 2-chloroethylamine hydrochloride, m.p. 140–142°, was 98.5%. Yields of crude product of 99% and 97% have been reported with use of chloroform[4] and toluene,[5] respectively, as suspension media. 2-Chloroethylamine hydrochloride melted at 148.5–150° after recrystallization from ethanol.[5]

5. This procedure is an adaptation of that described by Wystrach.[5]

6. The sodium hydroxide pellets are not placed under vacuum until they are cooled with liquid nitrogen because of their water content.

7. This procedure is an adaptation of that outlined by Kuh.[6]

8. The crude product still contains triethylamine hydrochloride.

9. A viscous noncrystalline residue is insoluble in hexane.

10. A radioautograph of the paper strip chromatogram of the product showed the presence of only one labeled component.

[1]S. Monat, C. Robbins and A. R. Ronzio, Atomic Energy Commission Report, AECU-672.

[2]J. D. Cox and R. J. Warne, J. Chem. Soc., *1951*, 1893.

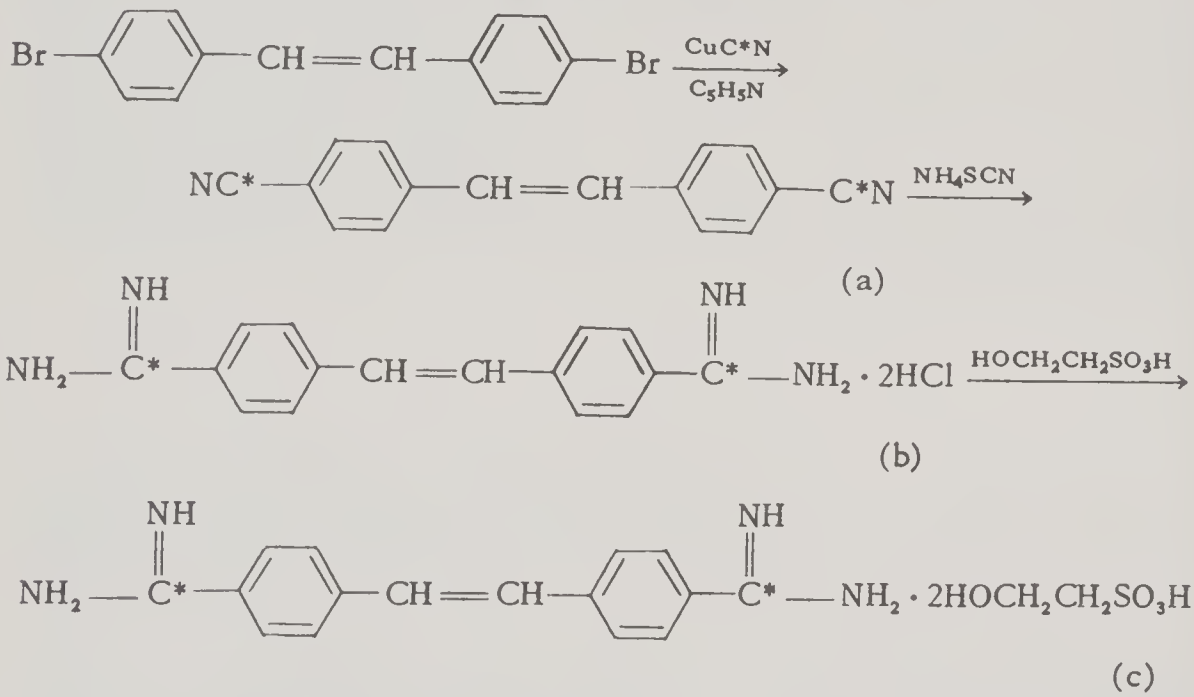
[3]L. O. Pilgeram, E. M. Gal, E. N. Sassenrath and D. M. Greenberg, J. Biol. Chem., *204*, 367 (1953).

[4]G. W. Raiziss and L. W. Clemence, J. Am. Chem. Soc., *63*, 3124 (1941).

[5]V. P. Wystrach, D. W. Kaiser and F. C. Schaefer, *ibid.*, *77*, 5915. (1955).

[6]E. Kuh, N. Brunswick and D. R. Seeger, U. S. 2,670,347, Feb. 23, 1954.

trans-4,4′-STILBENEDICARBOXAMIDINE-C_2^{14} DIISETHIONATE (C_2^{14}-Stilbamidine Diisethionate)



E. L. Bennett, D. E. Pack, B. J. Krueckel and J. C. Weaver, Cancer Research, *13*, 30 (1953).

A. Procedure (Note 1)

(a) trans-*4,4′-Stilbenedicarbonitrile-*C_2^{14}. A mixture of 0.349 g. (3.88 mmoles) of cuprous cyanide-C^{14}, 0.657 g. (1.94 mmoles) of *trans*-4,4′-dibromostilbene, 0.045 g. of cupric sulfate and 5 ml. of pyridine (distilled from barium hydroxide) is heated with shaking for 2 hours at 250° in a sealed tube (15 × 180 mm.). The tube is cooled in liquid nitrogen and opened, and the contents are treated with 10–15 volumes of cold 6 *N* hydrochloric acid. The product is filtered off, washed with acid, water, and a small amount of cold absolute ethanol, then air-dried. The yield is 0.452 g. of crude product, 101% (Note 2).

(b) trans-*4,4′-Stilbenedicarboxamidine-*C_2^{14} *Dihydrochloride.* A mixture of 0.440 g. of the dinitrile and 1.5 g. of dried ammonium thiocyanate is

heated in a sealed tube at 185 ± 5° for 14 hours. The tube is cooled in liquid nitrogen and opened, and the mixture is extracted with 7–8 ml. of cold 0.5 *N* ammonium hydroxide. The precipitate is extracted with 10 ml. of *N* hydrochloric acid at 80°, then with 20-ml. portions of warm water, followed by acid and water, until the volume of combined filtrate is 100 ml. The solution is decolorized with 0.020 g. of Norit, then made 2 *N* in hydrochloric acid and stored overnight at 0° to crystallize the product.

(c) trans-*4,4′-Stilbenedicarboxamidine-*C_2^{14} *Diisethionate* (Note 3). The crude dihydrochloride is dissolved in 10 ml. of water and colorized with charcoal; then the filtrate is cooled and made alkaline with 6 *N* ammonia to precipitate the free base. The precipitate is centrifuged, washed once with a little cold water and dissolved, without delay, in a solution of 0.63 g. of isethionic acid and 2 ml. of water. The product is precipitated by diluting with 20 volumes of acetone. Purification is effected by dissolution in water and reprecipitation with acetone; yield 0.315 g., 32% based on cuprous cyanide (Note 4). The molar specific activity is the same as that of the starting material, and both the infrared spectrum and paper chromatography indicate that the compound is pure and identical to an authentic sample.

B. Notes

1. The procedure is a modification of that used in the isotopic synthesis reported by Reid and Weaver,[1] which was based on the method of Bance.[2]

2. The pure compound melts at 282°.

3. The diisethionate salt is prepared because of its high solubility of 1 g. in 3 ml. of water. Isethionic acid may be prepared by the method of Goldberg.[3] An aqueous solution of barium isethionate, prepared from ethyl sulfate and fuming sulfuric acid, is neutralized with sulfuric acid, the clear solution is evaporated, and the deliquescent residue is dried under high vacuum.

4. Yields of 50–55% have been obtained in other preparations. The compound melts with gas evolution at 308–312°. The crystals are discolored by light. Neutral or alkaline solutions are unstable, the thermal stability of aqueous solutions increasing with lower pH.

C. Other Preparations

trans-4,4′-Stilbenedicarboxamidine-C_2^{14} diisethionate has been prepared by Reid[1] in 19% yield based on sodium cyanide. *trans*-4,4′-Stilbenedicarbonitrile-C_2^{14} was formed in the described manner (104% yield), then converted to methyl *trans*-4,4′-stilbenedicarboximidate-C_2^{14} dihydrochloride (79% yield) by shaking for 60 hours in a sealed tube with a chloroform-methanol solution saturated with hydrogen chloride. The imido-ester

hydrochloride was converted to *trans*-4,4′-stilbenedicarboxamidine-C_2^{14} (96% crude yield) by heating for 36 hours at 60° with a 10% solution of anhydrous ammonia in absolute alcohol. Purification was accomplished by forming the dihydrochloride and reprecipitating the free base with ammonia. The diisethionate then was formed in the manner described; yield 34% based on the imido-ester dihydrochloride, m.p. 308–312° (dec.).

[1]J. C. Reid and J. C. Weaver, Cancer Research, *11*, 188 (1951).

[2]S. Bance, H. J. Barber and A. M. Woolman, J. Chem. Soc., *1943*, 1; J. N. Ashley, H. J. Barber, A. J. Ewins, G. Newberry and A. D. H. Self, *ibid.*, *1942*, 103.

[3]A. A. Goldberg, J. Chem. Soc., *1942*, 716.

BIS(ACETIC-1-C^{14}) ANHYDRIDE

$$CH_3C^*OONa \xrightarrow{p\text{-}CH_3C_6H_4SO_2Cl} (CH_3C^*O)_2O$$

H. G. Mandel, Department of Pharmacology, The George Washington University School of Medicine, Washington, D. C.

A. Procedure (Note 1)

Anhydrous sodium acetate-1-C^{14} (2.0 g., 24 mmoles, Note 2) is ground to a fine powder in a mortar with 8.5 g. (45 mmoles) of *p*-toluenesulfonyl chloride. The mixture is heated with a bath at 200–260°, and the distilled product is collected in a receiver cooled with an ice-salt mixture (Note 3); yield 1.0 g. (80%).

B. Notes

1. The procedure is adapted from the method described in a patent[1] and used by Schantz[2] in the prepatation of bis(acetic-1-C^{14}) anhydride, 91% yield at the 22-mmole level.

2. The salt is dried over Drierite under vacuum at 130° in an Abderhalden drying pistol.

3. After brief cooling of the system, heating is resumed to distill some acetic anhydride which recondenses into the reaction flask. Macdonald and Baker[3] heated a mixture of 0.6080 g. of sodium acetate-1-C^{14} and 1.62 g. of *p*-toluenesulfonyl chloride at 180–200° for 30 minutes, then distilled the product; radiochemical yield 89%. Bruce[4] heated a similar mixture at 100–130° for 2 hours, then collected the product by distillation at 100° (25 mm.) into a trap at −79°, sweeping the apparatus with carrier.

C. Other Preparations

Bis(acetic-2-C^{14}) anhydride is prepared[5] in a similar manner from sodium acetate-2-C^{14}.

Bis(acetic-1-C^{14}) anhydride has been prepared by exchange between dry salts of acetic-1-C^{14} acid and acetic anhydride, with the product isolated by distillation. Ruben[6] has reported 55% and 62% of random distribution obtained with sodium acetate-1-C^{14} at room temperature in 20 and 30 minutes, respectively. Evans,[7] carefully studying a series of isotopic exchange reactions in anhydrous acetic acid or acetic anhydride solvent, reported only 4.6% exchange under similar heterogeneous conditions. In a homogeneous system, however, the exchange between plumbic acetate and acetic anhydride was complete within a few minutes. The exchange between acetyl chloride and acetic anhydride was found to be very slow. Several diagrams of apparatus and descriptions of the technique are given by Evans. Avivi[8] has brought the exchange to completion by heating 0.0312 g. of fused sodium acetate-1-C^{14} with 0.300 g. of acetic anhydride for 4 hours in a sealed tube at 140°. The product was vacuum-distilled in 40% yield. Milhaud[9] has prepared the anhydride by storing a mixture of 0.005 g. of sodium acetate-1-C^{14} and 10 μl. of acetic anhydride at room temperature for 24 hours. The liquid was assayed as acetanilide-1-C^{14}.

Bis(acetic-1-C^{14}) anhydride has been formed,[10] presumably as a by-product in the preparation of acetyl-1-C^{14} chloride, by an exchange reaction between acetyl chloride and sodium acetate-1-C^{14}.

[1]Chemische Fabrik von Heyden, Aktien-Gessellschaft, D. R. P. 123,052; Chem. Zentr., *72*, 518 (1901).

[2]E. M. Schantz and D. Rittenberg, J. Am. Chem. Soc., *68*, 2109 (1946).

[3]R. Macdonald and B. E. Baker, Can. J. Chem., *31*, 517 (1953).

[4]A. K. Bruce, Atomic Energy Commission Report, UR-339.

[5]N. S. Radin, D. Rittenberg and D. Shemin, J. Biol. Chem., *184*, 745 (1950).

[6]S. Ruben, M. B. Allen and P. Nahinsky, J. Am. Chem. Soc., *64*, 3050 (1942).

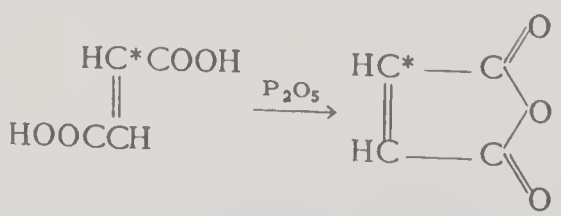
[7]E. A. Evans, J. L. Huston and T. H. Norris, J. Am. Chem. Soc., *74*, 4985 (1952).

[8]P. Avivi, S. A. Simpson, J. F. Tait and J. K. Whitehead, *Radioisotope Conference, 1954*, Vol. I, Academic Press, New York, 1954, p. 313.

[9]G. Milhaud, A. A. Benson, R. C. Fuller, V. Milhaud and M. Calvin, Atomic Energy Commission Report, UCRL-2934; Nuc. Sci. Abstracts, *9*, 4973 (1955).

[10]G. L. Curran, J. Biol. Chem., *191*, 775 (1951).

MALEIC-2-C^{14} ANHYDRIDE



R. F. Nystrom, Y. H. Loo and J. C. Leak, J. Am. Chem. Soc., 74, 3434 (1952).

A. Procedure (Note 1)

The apparatus consists of a 50-ml. round bottomed flask (attached to a vacuum manifold by way of a small U-type trap), a small collection trap and a stopcock. The flask contains 0.804 g. (6.93 mmoles) of fumaric-2-C^{14} acid between two layers of phosphorus pentoxide (4 g. each). The system is evacuated to 1 mm. and isolated from the pump.

The reaction flask is completely immersed in an oil-bath, and the temperature is raised to 155–160° for 3 hours. The crude product is collected by cooling the U-type trap with liquid nitrogen and then sealing off the reaction flask. The product is vacuum-distilled, at a bath temperature of 100°, into the collection trap which is cooled with liquid nitrogen; yield 0.651 g. (96%), m.p. 53.5–54° (Note 2). Purity is established by forming a derivative having the same molar specific activity as the fumaric-2-C^{14} acid (and succinic-2-C^{14} acid) starting material (Note 3).

B. Notes

1. The procedure is adapted from the method of Tanatar.[1]

2. An aliquot furnished a neutralization equivalent of 49.5 (theory 49.0).

3. 4-Cyclohexene-1,2-dicarboxylic-1-C^{14} anhydride, (*cis*-Δ^4-tetrahydrophthalic-1-C^{14} anhydride), m.p. 104.5–105.5°, is prepared[2] almost quantitatively by a Diels Alder addition of butadiene and maleic-2-C^{14} anhydride. *cis*-Δ^4-Tetrahydrophthalic-1,2-C_2^{14} anhydride,[3] prepared from maleic-2,3-C_2^{14} anhydride, was converted to phthalic-1,2-C_2^{14} acid. The mercuric salt was refluxed in hydrochloric acid at 140° for 3 hours, and benzoic-1,2-C_2^{14} acid was isolated in 90% yield.[3]

C. Other Preparations

Maleic-2,3-C_2^{14} anhydride has been prepared by Nystrom[3] in 70% over-all purified yield. A mixture of fumaric-2,3-C_2^{14} acid, acetyl chloride, acetic anhydride and glacial acetic acid was heated at 140–145° for 24 hours in a sealed tube. Purification was accomplished by sublimation.

The preparation of maleic-1-C^{14} anhydride (from sodium cyanide-C^{14}) by a modification of the described procedure has been reported by Lester.[4] The anhydride was subjected to the Diels Alder reaction with 1-phenyl-1,3-butadiene, 1-(*p*-methoxyphenyl)-1,3-butadiene and 1-(*p*-nitrophenyl)-1,3-butadiene at room temperature to determine the relative values of the specific reaction rate constants for the three dienes. The

respective products, 3-phenyl-, 3-(*p*-methoxyphenyl)- and 3-(*p*-nitrophenyl)-4-cyclohexene-1,2-dicarboxylic-$C^{14}_{1/2}$ anhydride, were determined by isotope dilution analysis.

[1]S. Tanatar, Ann., *273*, 31 (1893).
[2]E. H. Farmer and F. L. Warren, J. Chem. Soc., *1929*, 897; *Organic Reactions*, Vol. IV, Wiley, New York, 1948, p. 15.
[3]R. F. Nystrom, Y. H. Loo, K. M. Mann and J. R. Allen, Nucleonics, 7 (3), 58 (1950); 7 (4), 46 (1950).
[4]C. T. Lester and G. A. Ropp, Anal. Chem., *25*, 1933 (1953).

ETHYL FORMATE-C^{14}

$$HC^*OONa \xrightarrow{PO(OC_2H_5)_3} HC^*OOC_2H_5$$

J. G. Burr, Jr., J. Am. Chem. Soc., 73, 823 (1951).

A. Procedure

A mixture of 0.7 g. of dry sodium formate-C^{14} and 5 ml. of vacuum-distilled ethyl phosphate is heated to 170–210° for 30 minutes under reflux (Note 1). The mixture is cooled to room temperature; then the upper end of the reflux condenser is attached to a vacuum manifold through a trap cooled to −18° and a second trap cooled with liquid nitrogen. The product is distilled at high-vacuum and collected in the low-temperature receiver. Redistillation at 10 mm. into a graduated tube yields 0.80–0.82 ml. of ester, 0.770 g., having a vapor pressure of 77.5 at 0°. Both the chemical and radiochemical yields are quantitative.

B. Notes

1. The procedure is adapted from that described by Ropp; see ethyl acetate-2-C^{14} and butyl acetate-2-C^{14}. The salt is dried for several hours at 100–120° at 0.5 μ. Pichat[1] has reported that quantitative yields are obtained with either ethyl sulfate or the phosphate. The sulfate is heated at 155–160°; see indole-2-C^{14} (Note 3).

C. Other Preparations

Ethyl formate-C^{14} has been prepared in 69–98% yield by Burr,[2] by a similar procedure employing ethyl sulfate. Considerable contamination

with a volatile impurity was observed when the temperature was raised above 185°.

Methyl formate-C^{14} ($n_D^{6.5}$ 1.3508) has been prepared similarly by Melville,[3] employing methyl sulfate and heating for 2 hours at 185° in a slow stream of nitrogen; a photograph of the apparatus is presented. A similar preparation has been described by Bernhard.[4]

Ethyl formate-C^{14} has been prepared by van den Bos[5] by refluxing for 2 hours a mixture of the acid, the calculated amount of ethanol, and sulfuric acid. The ester was isolated by distillation and purified by redistillation, b.p. 53–57°. Pyrolysis of the product at 500° yielded isotopic carbon dioxide and nonisotopic ethylene.

Ethyl formate-$C^{14}_{1/1}$-$H^2_{1/1}$ has been prepared[6] in a similar manner by distilling, through an efficient fractionating column, a mixture of 1 mole of formic-H^2 acid and 0.8 mmole of sodium formate-C^{14} in 1500 ml. of absolute alcohol containing a few drops of concentrated sulfuric acid. The main fraction of product distilled at 52–54°.

Ethyl formate-C^{14} has been prepared by Dische[7] in 90% yield based on barium carbonate. An ethereal extract of formic-C^{14} acid was esterified with ethereal diazoethane.

[1]L. Pichat, M. Audinot and J. Monnet, Bull. soc. chim. France, (5) *21*, 85 (1954).

[2]J. G. Burr, Jr., W. G. Brown and H. E. Heller, J. Am. Chem. Soc., *72*, 2560 (1950); Nucleonics, 7 (3), 54 (1950).

[3]D. B. Melville, J. R. Rachele and E. B. Keller, J. Biol. Chem., *169*, 419 (1948); C. Graebe, Ann., *340*, 244 (1905).

[4]K. Bernhard, G. Brubacher and A. H. Lutz, Helv. Chim. Acta, *37*, 1839 (1954).

[5]B. G. van den Bos and A. H. W. Aten, Jr., Rec. trav. chim., *70*, 495 (1951).

[6]D. Elwyn, A. Weissbach, S. S. Henry and D. B. Sprinson, J. Biol. Chem., *213*, 281 (1955).

[7]R. Dische and D. Rittenberg, J. Biol. Chem., *211*, 199 (1954).

ETHYL ACETATE-2-C^{14}

$$CH_3C^*OONa \xrightarrow{(C_2H_5O)_3PO} CH_3C^*OOC_2H_5$$

G. A. Ropp, J. Am. Chem. Soc., *72*, 2299 (1950).

A. Procedure

A mixture of 0.406 g. (4.95 mmoles) of anhydrous sodium acetate-2-C^{14}, dried for several hours at 110–120° at 0.5 μ, and 1.5 ml. of distilled ethyl phosphate (Note 1) is heated at 170–220° for 1 hour under reflux in

a 10-ml. pear-shaped flask containing a small piece of glass wool. The mixture is cooled, and the upper end of the condenser is attached to a vacuum manifold through a trap at −18° and a second trap cooled with liquid nitrogen. The mixture is distilled at 70° at 0.1–0.01 μ, with a small amount of ethyl phosphate being collected in the first trap and the product in the second; yield 0.400 g., 92% (Note 2).

Butyl acetate-2-C^{14} is prepared in 82% yield by a similar procedure employing butyl phosphate. The product is collected by distillation at 0.5 μ by heating for 2 hours at 80–140°. Cold running water in the vertical condenser replaces the first trap. The purity is checked by dilution technique (Note 3).

B. Notes

1. The alkyl phosphates are preferable to the sulfates for the esterification of acetic acid, because of higher boiling point and greater stability of the reagent at 200°. See ethyl formate-C^{14}.

2. The vapor pressure is 31 mm. at 0°, 95 mm. at 20°, and 122 mm. at 30°. Fields[1] has reported a 95% yield.

3. An aliquot of butyl acetate-2-C^{14} is diluted 100-fold with pure butyl acetate, and a 2.0-ml. aliquot is heated under reflux at 100–130° for 3.5 hours with 4 ml. of benzylamine[2] and 4 ml. of water. The mixture is distilled at 15 mm. (bath temperature 190°) until only a brown solid remains. The residue is twice recrystallized from ligroin (b.p. 90–120°) with charcoal treatment. The yield of *N*-benzylacetamide-2-C^{14} is 0.5 g., m.p. 60–61.5°. The molar specific activity is essentially that calculated from the sodium acetate-2-C^{14}, thus indicating the essential purity of the ester.

C. Other Preparations

Ethyl acetate-1-C^{14} has been prepared in 95% yield by Fields[1] and Pearce[1a] (apparatus diagram) by the described procedure.

The preparation of ethyl acetate-1-C^{14} (-1-C^{13} or -2-C^{14}), by a similar procedure employing ethyl sulfate, has been described by Tolbert[3] (95–97%), Fields[4] (85%), Sakamii (quantitative), Weinhouse[6] (92%), Lemmon[7] (90%), Copper,[8] Langdon[9] and in detail by Cox and Turner[10] (102–120% yield).

Methyl acetate-1-C^{13} has been prepared in nearly quantitative yield by Sakami,[5] by employing methyl sulfate.

The preparation of ethyl acetate-1-C^{14} (90% yield) by refluxing a mixture of 0.5 g. of barium acetate-1-C^{14}, concentrated sulfuric acid and 2 molar amounts of ethanol has been described by Neyman.[11]

Ethyl propionate-3-C^{14} has been prepared[12] by reacting 0.278 mmoles of powdered sodium propionate-3-C^{14} with 2.0 ml. of ethyl phosphate at 170–185° in an apparatus similar to that described by Melville,[13] the product being collected in a spiral trap with a sintered glass plate (see Figure X, 1).

Ethyl crotonate-1-C^{14} has been prepared[14] by heating for 1 hour at 180–210° a mixture of 3.8 ml. of ethyl phosphate (b.p. 104–105° at 20 mm.) and the sodium salt prepared by titration of 1.1 g. of crotonic-1-C^{14} acid and dried under vacuum at 120°; yield of vacuum-distilled product 1.3 g., n_D^{20} 1.4247. Preparation by esterification with ethanol in the presence of sulfuric acid has been reported by Swan.[15]

[1]M. Fields, M. A. Leaffer, S. Rothchild and J. Rohan, J. Am. Chem. Soc., *74*, 5498 (1952).

[1a]G. W. Pearce and J. A. Jensen, J. Agr. Food Chem., *1*, 776 (1953).

[2]C. A. Buehler and C. A. Mackenzie, J. Am. Chem. Soc., *59*, 421 (1937).

[3]B. M. Tolbert, F. A. Christenson, F. N. H. Chang and P. P. T. Sah, J. Org. Chem., *14*, 525 (1949).

[4]M. Fields, M. A. Leaffer and J. Rohan, Science, *109*, 35 (1949).

[5]W. Sakami, W. E. Evans and S. Gurin, J. Am. Chem. Soc., *69*, 1110 (1947).

[6]S. Weinhouse, G. Medes and N. F. Floyd, J. Biol. Chem., *166*, 691 (1946).

[7]R. M. Lemmon, Atomic Energy Commission Report, AECU-452; Nuc. Sci. Abstracts, *3*, 1626 (1949).

[8]C. Cooper, R. Wu and D. W. Wilson, J. Biol. Chem., *216*, 37 (1955).

[9]R. G. Langdon and K. Bloch, J. Biol. Chem., *200*, 135 (1953).

[10]J. D. Cox and H. S. Turner, J. Chem. Soc., *1950*, 3176.

[11]M. B. Neyman and G. I. Feklisov, Doklady Akad. Nauk S.S.S.R., *87*, 605 (1952); Nuc. Sci. Abstracts, *7*, 4095 (1953); AEC-tr-1828.

[12]R. B. Henderson, R. M. Fink and K. Fink, J. Am. Chem. Soc., *77*, 6381 (1955).

[13]C. Melville, J. Rachele and E. Keller, J. Biol. Chem., *169*, 419 (1947).

[14]O. Simamura, N. Inamato and T. Suehiro, Bull. Chem. Soc. Japan, *27*, 221 (1954); through Chem. Abstracts, *49*, 7494 (1955).

[15]G. A. Swan, J. Chem. Soc., *1955*, 1039.

PHENYL ACETATE-1-C^{14}

$$CH_3C^*OONa \xrightarrow{SOCl_2} CH_3C^*OCl \xrightarrow{C_6H_5OH} CH_3C^*OOC_6H_5$$

R. B. Turner, J. Am. Chem. Soc., *72*, 579 (1950).

Procedure

To a stirred mixture of 2.85 g. of anhydrous sodium acetate-1-C^{14} and 10 ml. of dry benzene is added slowly 5.0 g. (1.2 equivalents) of

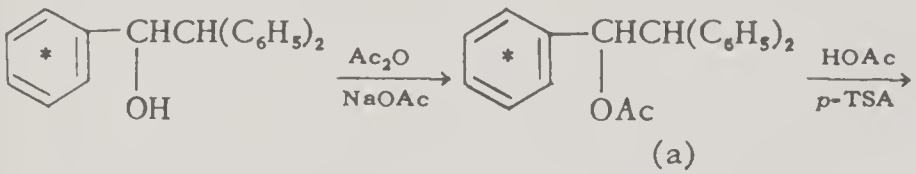
thionyl chloride in 5 ml. of benzene with ice-bath cooling. When the evolution of sulfur dioxide subsides (10 minutes), the mixture is stored at room temperature for 1.5 hours. The mixture is refluxed with 5.0 g. of phenol for 4.5 hours, then cooled, diluted with water and extracted with ether. The ether solution is washed with dilute sodium hydroxide, dried and fractionated; yield 4.32 g. (91 %), b.p. 82.5–83° (14 mm.), n_D^{25} 1.5038.

By the same procedure is prepared phenyl acetate-2-C^{14} from sodium acetate-2-C^{14}.

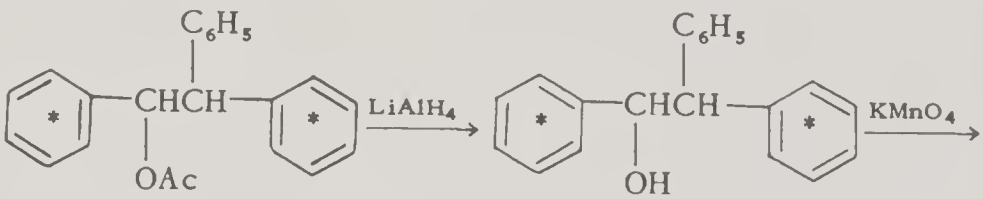
The use of this procedure has been reported by Gut.[1]

[1]M. Gut, Helv. Chim. Acta, 36, 906 (1953).

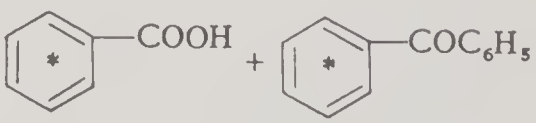
1,2-BIS(PHENYL-$C^{14}_{1/4}$)-2-PHENYLETHYL ACETATE



(a)



(b)



C. J. Collins and W. A. Bonner, J. Am. Chem. Soc., 77, 92 (1955).

A. Procedure (Note 1)

(a) *1-Phenyl-$C^{14}_{1/4}$-2,2-diphenylethyl Acetate.* 1-Phenyl-$C^{14}_{1/4}$-2,2-diphenylethanol is acetylated in the manner described by Bonner[1] for the chain-labeled analog, which follows. Degradation studies indicate that there is no rearrangement.

A mixture of 1.00 g. of 1,2,2-triphenylethanol-1-C^{14}, 10 ml. of hot glacial acetic acid, 0.2 g. of anhydrous sodium acetate and 5 ml. of acetic

anhydride is heated on a steam-bath for 16 hours, then diluted with water slightly and set aside to crystallize. The yield of 1,2,2-triphenylethyl-1-C^{14} acetate is 1.13 g. (99%), m.p. 156.5–157°.

(b) *1,2-Bis(phenyl-$C^{14}_{1/4}$)-2-phenylethyl Acetate* (Note 2). To a solution of 1.46 g. (4.62 mmoles) of 1-phenyl-$C^{14}_{1/4}$-2,2-diphenylethyl acetate in 17 ml. of warm acetic acid is added 0.88 g. (5.0 mmoles) of *p*-toluenesulfonic acid hydrate and 0.5 ml. (4.9 mmoles) of acetic anhydride. The mixture is heated for 1.25 hours in a steam-bath, diluted slightly with water and set aside to crystallize; yield 1.13 g. (78%). Recrystallization from acetic acid gives 0.94 g. of pure product, m.p. 153–154°. Degradation studies indicate that 63.7% of the activity is located at 2-phenyl (Note 3).

B. Notes

1. These preparations are incidental to a study of the mechanism of the Wagner-Meerwein rearrangement in the 1,2,2-triphenylethyl system; see 1,2,2-triphenylethanol-$C^{14}_{1/2}$. Kinetic studies with the acetate and *p*-toluenesulfonic acid have been reported by Bonner.[2]

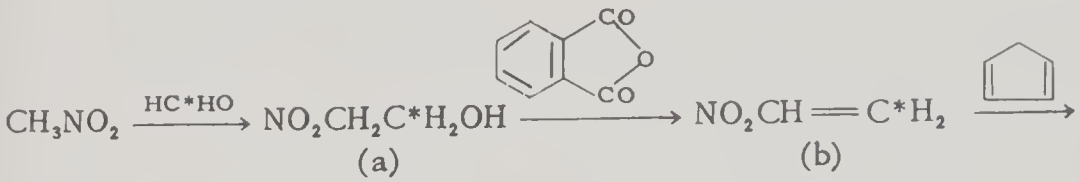
2. See 1,2,2-triphenylethanol-$C^{14}_{1/2}$, Note 12.

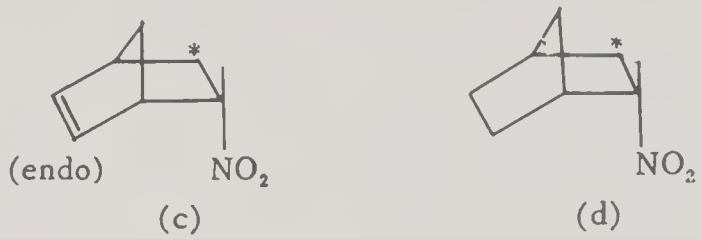
3. The acetate was deacetylated with lithium aluminum hydride in ether (see 1,2,2-triphenylethanol-$C^{14}_{1/2}$), forming 1,2-bis(phenyl-$C^{14}_{1/4}$)-2-phenylethanol (0.88 g. yield), which was oxidized with permanganate, forming benzoic-1,2,3,4-$C^{14}_{1/4}$ acid and benzophenone-1,2,3,4-$C^{14}_{1/4}$. The molar specific activities of the two degradation products were 36.3% and 63.7%, respectively, of that of the starting material. As with the acetolysis of 1,2,2-triphenylethyl-1-C^{14} acetate, the distribution is essentially statistical.

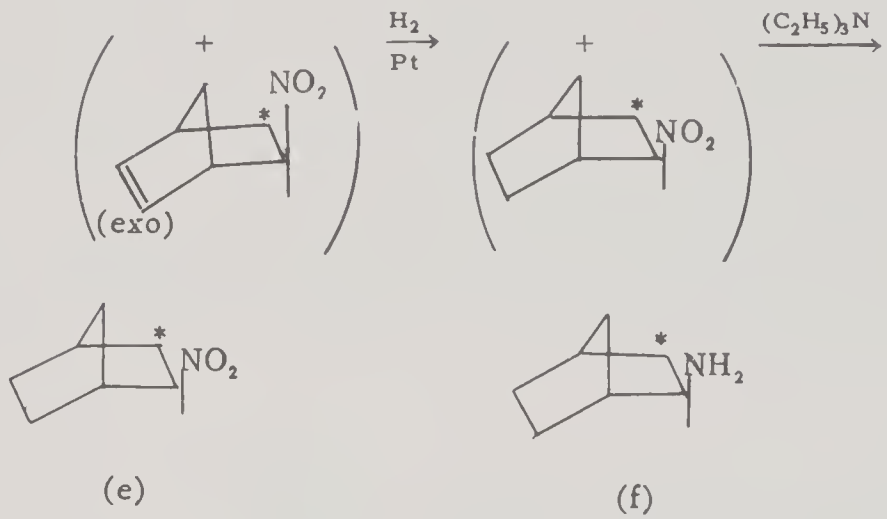
The degradation of benzophenone-$C^{14}_{1/1}$-1,2,3,4-$C^{14}_{1/4}$, obtained by isomerization of a mixture of ring- and chain-labeled 1,2,2-triphenylethyl acetates, is described by Bonner.[2] The ketone is refluxed with pyridine and hydroxylamine hydrochloride in alcohol for 24 hours, then poured into water and filtered. The benzophenone-$C^{14}_{1/1}$-1,2,3,4-$C^{14}_{1/4}$ oxime is subjected to the Beckmann rearrangement by warming with concentrated sulfuric acid for 1 hour on a steam-bath. The mixture is poured onto ice and extracted with ether, yielding benzoic-$C^{14}_{1/1}$-1,2,3,4-$C^{14}_{1/4}$ acid; aniline-$C^{14}_{1/4}$ is the other product.

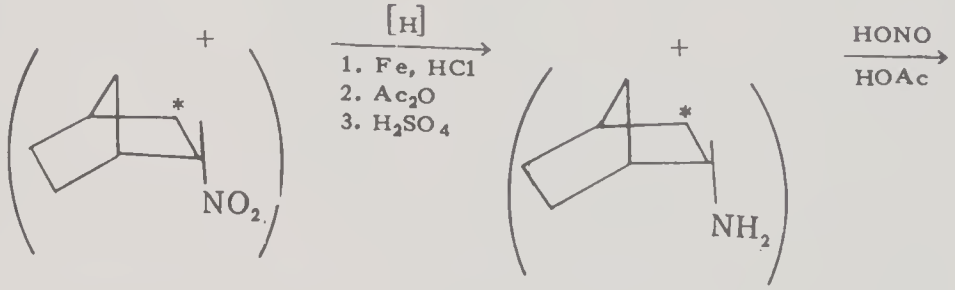
[1]W. A. Bonner and C. J. Collins, J. Am. Chem. Soc., 75, 5372 (1953).
[2]W. A. Bonner and C. J. Collins, J. Am. Chem. Soc., 77, 99 (1955).

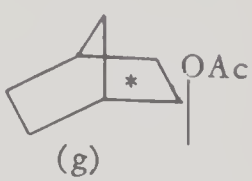
C_1^{14}-*exo*-2-NORCAMPHANYL ACETATE
(C_1^{14}-*exo*-Norbornyl Acetate)











J. D. Roberts, C. C. Lee and W. H. Saunders, Jr., J. Am. Chem. Soc., 76, 4501 (1954); 77, 3034 (1955).

A. Procedure

(a) *2-Nitroethanol-1-C^{14}*. Figure III, 2 is a diagram of the apparatus used (Note 1). A mixture of 100 g. of redistilled nitromethane (b.p. 100–101°), 40 g. of dioxane and 1.5 ml. of 10% citric acid solution (Note 2) is heated under reflux in the round-bottomed flask at 120–130° (175 mm.). To the stirred nitromethane in the reaction flask is added, dropwise during 2 hours, a mixture of 12 g. of 36% formalin, 0.133 mmole of formaldehyde-C^{14} in 4.5 ml. of water, 16 g. of dioxane and 0.4 ml. of 10% sodium hydroxide solution. Finally, a mixture of 4 g. of 36% formalin, 4 g. of dioxane and 0.1 ml. of 10% sodium hydroxide is added during 20 minutes, and the refluxing is continued for 15 minutes. The yellow solution in the round-bottomed flask is filtered and evaporated under reduced pressure. The residue is diluted with 6.0 g. of carrier, and the product is fractionated, the still being scavanged with 13.0 g. of carrier in 3 portions; yield 29.0 g. (58% corrected), b.p. 59–63° (1.0 mm.), radiochemical yield 45%.

(b) *1-Nitroethylene-2-C^{14}*. 2-Nitroethanol-1-C^{14} is dehydrated with 90 g. of phthalic anhydride by the procedure of Buckley and Scaife[1], which follows. The product is collected in an ice-cooled receiver containing a few grams of anhydrous calcium chloride to minimize polymerization of the product (Note 3). A filtered dry ethereal solution (50 ml.) of the 1-nitroethylene-2-C^{14} is used in the next step.

A mixture of 100 g. of 2-nitroethanol and 180 g. of phthalic anhydride is heated at 140–150° (80 mm.) in a distillation apparatus with a short

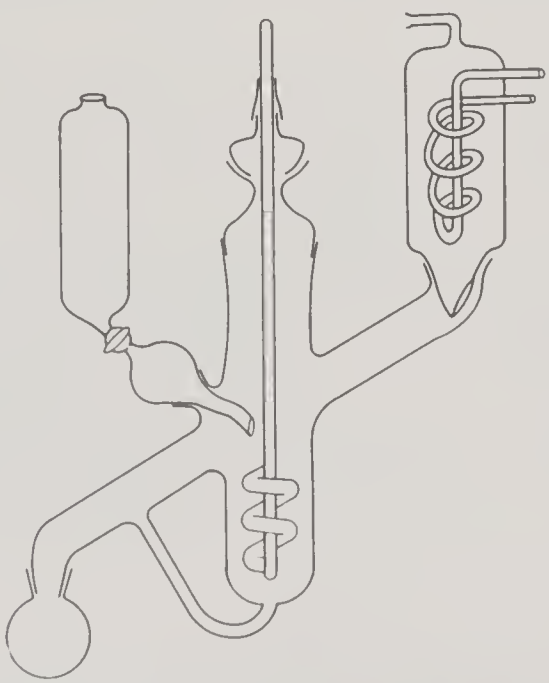


Fig. III, 2. Apparatus for condensation of formaldehyde and nitromethane (J. D. Roberts, C. C. Lee and W. H. Saunders, Jr.).

fractionating column. When the mixture becomes homogeneous, the bath temperature is raised to 175–180° until distillation ceases. The distillate is dried over calcium chloride and redistilled; yield 55 g. (66.5%), b.p. 38–39° (80 mm.).

(c) endo-6-*Nitrobicyclo*[2.2.1]*hept-2-ene-5-C*^{14}, (endo-5-*Nitrobornene-6-C*^{14}) (Note 4). To a stirred solution of 30 g. of freshly distilled, dry cyclopentadiene in 75 ml. of absolute ether is added the ethereal solution of 1-nitroethylene-2-C^{14} during 1.5 hours. The mixture is stored overnight and concentrated, and the product is distilled through a short vacuum-jacketed Vigreux column; yield 37.0 g. (61% based on 2-nitroethanol), b.p. 74–77° (4.3 mm.) (Note 5).

(d) endo-*2-Nitronorcamphane-3-C*^{14}, (endo-*2-Nitronorbornane-3-C*^{14}). The *endo*-5-Nitronorbornene-6-C^{14} is hydrogenated at 2 atmospheres over 0.2 g. of platinum oxide in 150 ml. of glacial acetic acid. Shaking is momentarily discontinued to permit cooling as necessary. The hydrogen absorption practically ceases after the calculated uptake. The filtered solution is concentrated under reduced pressure, and the residue is dissolved in 50 ml. of ether and washed with 25 ml. of water, then dried over magnesium sulfate. The solvent is evaporated, and the product is sublimed; yield 21.7 g., 81% (Note 6).

(e) exo-*2-Nitronorcamphane-3-C*^{14}, (exo-*2-Nitronorbornane-3-C*^{14}). A mixture of 32 g. of *endo*-2-nitronorbornane-3-C^{14} and 1.6 g. of triethylamine is heated overnight on a steam-bath. The mixture is dissolved in 100 ml. of pentane and stirred with 50 ml. of 20% acetic acid containing 10 g. of urea (Note 7). The pentane extract is dried over Drierite and concentrated, and the product is distilled; yield 31.0 g. (97%), b.p. 78° (4 mm.) (Note 8).

(f) exo-*2-Norcamphanamine-3-C*^{14}, (exo-*Norbornylamine-3-C*^{14}). To a mixture of 52 ml. of water, 52 ml. of dioxane and 14.2 ml. of concentrated hydrochloric acid is added 44.2 g. of iron filings (20 mesh). When gas evolution ceases, the mixture is treated with 31 g. of *exo*-2-nitronorbornane-3-C^{14} and refluxed for 2 days. A little additional hydrochloric acid is added, and 75 ml. of steam distillate is discarded. The residue is basified with sodium hydroxide; then 175 ml. of steam distillate is collected and extracted with 3 portions of ether. The combined extract is dried over potassium carbonate, then diluted to 31 ml. with absolute ether and added to a stirred solution of 22 g. of acetic anhydride in 300 ml. of ether during 30 minutes. The mixture is refluxed for 1 hour, washed with 10% potassium carbonate solution, then dried over potassium carbonate and concentrated on a steam-bath until solid appears. Systematic crystallization from ether and from heptane-ethyl acetate gives a total of 11.4 g. of *N*-(*exo*-2-norcamphanyl-3-C^{14})acetamide, m.p. 140–141° (Note 9).

A mixture of 11.0 g. of the *N*-acetyl derivative and 100 ml. of 10% sulfuric acid is refluxed for 24 hours. The solution is basified with sodium hydroxide and extracted with five 40-ml. portions of ether. The extract is dried over potassium carbonate and concentrated, and the product is distilled; yield 6.3 g. (79%), b.p. 78° (55 mm.) (Note 10). Degradation studies show that there is no isotopic rearrangement, see below.

(g)·C_1^{14}-exo-2-*Norcamphanyl Acetate.* To a stirred solution of 6.0 g. of *exo*-2-norcamphanamine-3-C^{14} in 40 ml. of glacial acetic acid is added 6.0 g. of sodium nitrite, portionwise during 1 hour. The mixture is stored overnight and treated with 1.5 g. of sodium nitrite (total 2 equivalents), then is diluted with 15 ml. of water and poured into 150 ml. of cold 20% sodium hydroxide. The mixture is extracted with five 40-ml. portions of pentane. The extract is washed with 1 *N* hydrochloric acid (Note 11), then dried over Drierite and concentrated, and the residue is distilled; yield 4.0 g., b.p. 77–79° (15 mm.). Degradation studies indicate that there is 48% isotopic rearrangement (Note 12).

B. Notes

1. The procedure is a modification of the method of Hayes[2] for the liquid phase condensation of formaldehyde with compounds containing activated hydrogens. Diagrams of apparatus are given by both Hayes and Roberts.

2. Neutralization of the alkaline catalyst with a nonvolatile acid minimizes the dehydration and polymerization reactions of the monomethylol derivatives which concentrate in the boiling flask.

3. Nitroethylene is moderately stable to heat and light, but polymerizes readily in the presence of water and violently in the presence of a trace of alkali.

4. The procedure is a modification of the method of Alder,[3] heating being unnecessary.

5. The product is semisolid at room temperature. There is no suitable method for determining the radio of *endo*- to *exo*-isomers. The stereochemical selectivity[4] of the reaction would lead one to expect the *endo*-form to predominate over 90%.

6. The melting point is not well defined, most of the material melting from 64–67°.

7. Urea in aqueous acetic acid is used to regenerate[5] nitroparaffins from their alkali salts. The efficiency of the reagent is due to the mild acidity, which minimizes the Nef reaction, and the rapidity with which it destroys the reactive nitrous acid.

8. The product of isomerization is a mixture of 70–80% *exo*- and 30–20% *endo*-isomers.

9. Repeated recrystallization of a small sample raises the melting point to 141–142.4°.

10. The compound solidifies in a refrigerator.

11. Less than 10% unreacted amine is recovered.

12. Directions are given by Roberts for degrading the product (see C_2^{14}-*cis*-1,3-cyclopentanedicarboxylic acid) through the series: C_1^{14}-*exo*-2-norcamphanol, C_1^{14}-*cis*-1,3-cyclopentanedicarboxylic acid, C_1^{14}-*cis*-1,3-cyclopentanediamine and *cis*-N,N′-(C_1^{14}-1,3-cyclopentylene)bisbenzamide. The molar specific activity of the amide is 48% of that of the acid.

C. Other Preparations

In a similar manner is prepared C_1^{14}-*exo*-2-norcamphanol by diazotization of *endo*-2-norcamphanamine-3-C^{14} in aqueous fluoboric acid; yield 77%, m.p. 117–121°. The infrared spectrum of the crude product indicates the presence of 2–5% of norcamphor.

From *endo*-6-nitrobicyclo[2.2.1]hept-2-ene-5-C^{14} is prepared *endo*-bicyclo [2.2.1]hept-5-en-2-amine-3-C^{14}. Diazotization in acetic acid (or aqueous fluoboric acid) gives a mixture of C^{14}-*exo*-tricyclo[2.2.1.$0^{2,6}$]heptanyl and C^{14}-*exo*-bicyclo[2.2.1]hept-5-enyl, (*exo*-dehydronorbornyl) acetates, (or alcohols); quantitative hydrogenation shows the dehydronorbornyl derivatives to be 17% (or 7%) of the product mixture. Degradation of the C^{14}-*exo*-2-norcamphanol indicates that there is 35–38.5% rearrangement of isotope.

In the same manner from *endo*-2-nitronorbornane-3-C^{14} are prepared *endo*-norbornylamine-3-C^{14} (70%), m.p. 53–59°, and *N*-(*endo*-2-norcamphanyl-3-C^{14})acetamide, m.p. 125–126° from ligroin (b.p. 90–100°).

[1]G. D. Buckley and C. W. Scaife, J. Chem. Soc., *1947*, 1471.

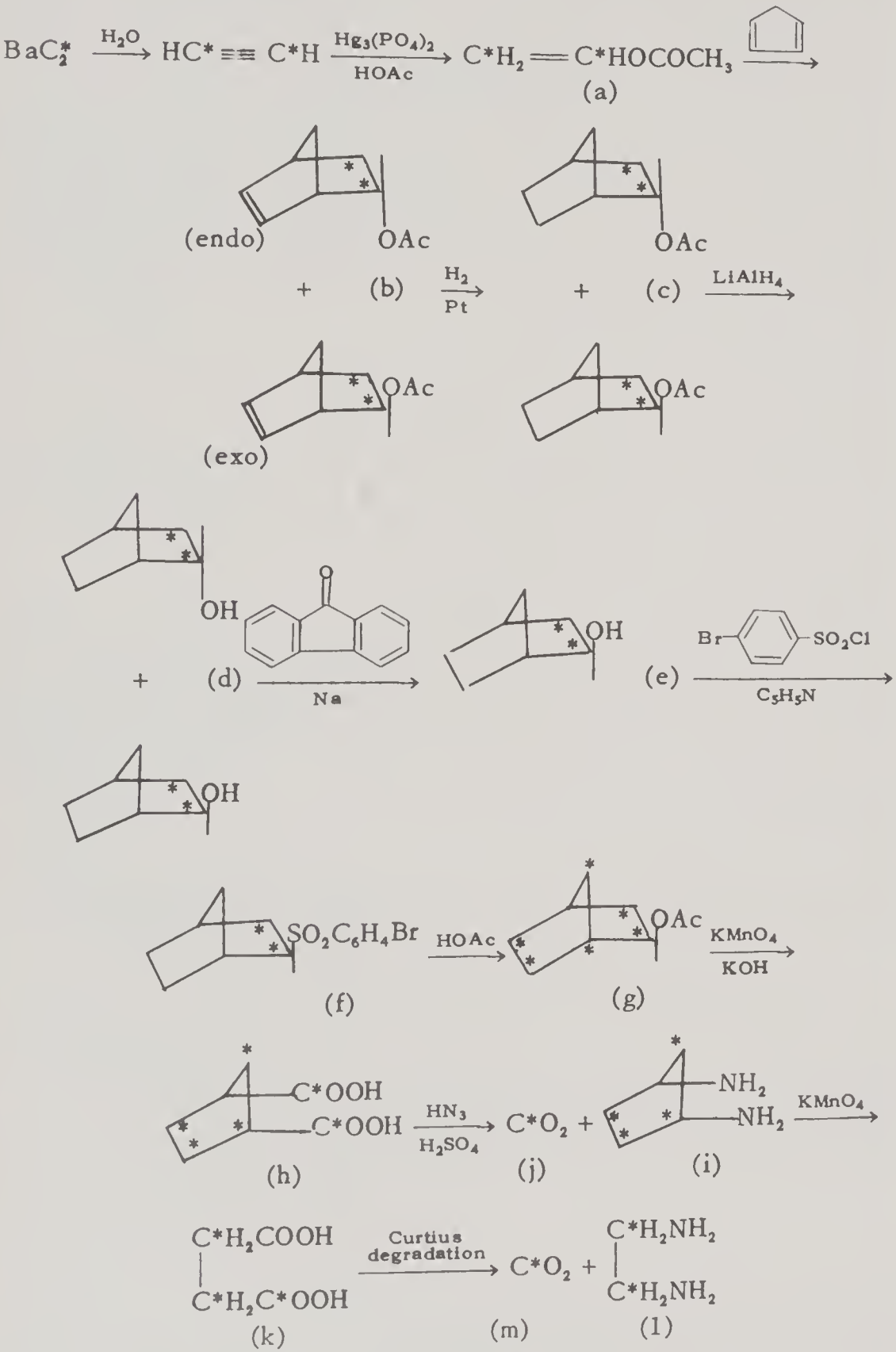
[2]J. T. Hayes, G. F. Hager, H. M. Engelmann and H. M. Spurlin, J. Am. Chem. Soc., 73, 5369 (1951); H. M. Spurlin, U. S. 2,510,914; Chem. Abstracts, *44*, P.7870 (1950).

[3]K. Alder, H. F. Rickert and E. Windemuth, Ber., *71*, 2451 (1938).

[4]*Organic Reactions*, Vol. IV, Wiley, New York, 1948, p. 10.

[5]N. Kornblum and G. E. Graham, J. Am. Chem. Soc., 73, 4041 (1951).

exo-2-NORCAMPHANYL-2,3-C_2^{14} *p*-BROMOBENZENESULFONATE
(*exo*-Norbornyl-2,3-C_2^{14} *p*-Bromobenzenesulfonate)



J. D. Roberts, C. C. Lee and W. H. Saunders, Jr., J. Am. Chem. Soc., 76, 4501 (1954); 77, 3034 (1955). J. D. Roberts and C. C. Lee, J. Am. Chem. Soc., 73, 5009 (1951).

A. Procedure

(a) *Vinyl-C_2^{14} Acetate* (Note 1). To a warm solution of 0.30 g. of mercuric oxide, 4.0 ml. of glacial acetic acid and 1.0 ml. of acetic anhydride, in a 1-l. flask, is added 5 ml. of a 5% solution of 1 : 1 phosphorus pentoxide-syrupy phosphoric acid in glacial acetic acid. The mixture, containing precipitated mercuric phosphate, is frozen with a Dry Ice-bath, and the flask is evacuated to 50 μ. The flask is filled with 1 l. of acetylene-C_2^{14}, closed off, and shaken overnight (Note 2). The product is removed with a pipette, then mixed with 1.0 g. of anhydrous potassium acetate and collected by distillation through a semimicro column; yield 2.73 g. (71%), b.p. 72–74° (Note 3).

(b) *Bicyclo[2.2.1]hept-2-en-6-yl-5,6-C_2^{14} Acetate*, (*Dehydronorbornyl-2,3-C_2^{14} Acetate*) (Note 4). A mixture of 14 g. of freshly distilled cyclopentadiene and 21 g. of vinyl-C_2^{14} acetate is heated at 200° for 10 hours in a sealed tube. The product is distilled through a Vigreux column; yield yield 13.5 g. (73% based on unrecovered vinyl acetate), b.p. 82–83° (17 mm.), n_D^{25} 1.4667. The radiochemical yield is 25% based on barium carbonate.

(c) *2-Norcamphanyl-2,3-C_2^{14} Acetate*, (*Norbornyl-2,3-C_2^{14} Acetate*). The bicyclo[2.2.1]hept-2-en-6-yl-5,6-C_2^{14} acetate is hydrogenated over platinum oxide in ethyl acetate at room temperature; yield 97%, b.p. 69° (6 mm.), n_D^{25} 1.4578 (Note 5).

(d) *2-Norcamphanol-2,3-C_2^{14}*, (*Norborneol-2,3-C_2^{14}*) (Note 6). 2-Norcamphanyl-2,3-C^{14} acetate is cleaved in ether solution with lithium aluminum hydride;[1] yield 90% (Note 7). The product is a mixture of *endo*- and *exo*-isomers (81 : 19).

(e) exo-*2-Norcamphanol-2,3-C_2^{14}* (Note 8). A mixture of 15 g. of 2-norcamphanol-2,3-C_2^{14}, 0.30 g. of 9-fluorenone, 0.15 g. of sodium and 35 ml. of toluene is refluxed for 48 hours. The solvent is evaporated under reduced pressure, and the residue is transferred into a sublimator with with methanol. The sublimed isomerization product, 13.2 g. (88%), contains 74% of the *exo*-isomer (Notes 7 and 9). Degradation studies (see below) show that there is no isotopic rearrangement.

(f) exo-*2-Norcamphanyl-2,3-C_2^{14}* p-*Bromobenzenesulfonate* (Note 10). The *exo*-2-Norcamphanol-2,3-C_2^{14} is reacted with *p*-bromobenzenesulfonyl chloride for 2 days at 0° in dry pyridine solution. The cooled solution is diluted with water and extracted at 0° with chloroform. The cold extract is washed with cold dilute sulfuric acid, water, and sodium bicarbonate, then is dried over sodium sulfate and evaporated under reduced pressure. The product is crystallized from ether-pentane; yield 80–85% (Note 11).

(g) C_2^{14}-exo-*2-Norcamphanyl Acetate* (Note 12). A solution of 7.9 g. of *exo*-2-norcamphanyl-2,3-C_2^{14} *p*-bromobenzenesulfonate, 2.5 g. of potassium acetate (10% excess) and 50 ml. of glacial acetic acid is heated

at 45° for 20 minutes. The solvent is evaporated under reduced pressure at 45°, and the residue is diluted with ice water, then basified with cold sodium hydroxide and extracted with ether. The extract is dried, concentrated (Note 13) and fractionally distilled, b.p. 60–61° (5 mm.). Degradation studies (see below) indicate that there is 60% isotopic rearrangement, the isotope distribution being C-1,4 (23%), C-2,3 (40%), C-5,6 (15%), C-7 (22%) (Note 14).

(h) C_2^{14}-cis-*1,3-Cyclopentanedicarboxylic Acid.* To a stirred solution of 1.0 g. of C_2^{14}-*exo*-norborneol (Note 15) in 5 ml. of pentane is added 4.0 g. of potassium permanganate and 40 ml. of cold 20% potassium hydroxide. The mixture is cooled with an ice-bath and stirred for about 3 hours, then saturated with sulfur dioxide to remove the manganese dioxide and excess permanganate. The mixture is extracted with eight 25-ml. portions of ether, and the combined extract is dried over magnesium sulfate, then concentrated to 10 ml. on a steam-bath and evaporated under reduced pressure at room temperature (Note 16). The solid residue is dissolved in ether and decolorized with carbon, and the product is precipitated by the addition of pentane; yield 40–70%, m.p. 120–121°. Degradation studies (Note 17) indicate that the distribution of isotope is that described for the previous compound.

B. Notes

1. The procedure is based on that of Morrison[2] and Groth.[3]

2. The pressure falls from 1 atmosphere to 60 mm.

3. The radiochemical yield may be raised by scavanging the still with 5 g. of carrier vinyl acetate, and by treating the reaction residue with 10 ml. of the catalyst mixture and 11. of carrier acetylene as described.

4. The procedure is an adaptation of that of Alder.[4,5] The Diels-Alder addition is not stereospecific,[5] for the product is a mixture of *exo*- and about 85% *endo*-isomers.

5. *endo*-Isomer, n_D^{25} 1.4583; *exo*-isomer, n_D^{25} 1.4565,[6] b.p. 60–61° (5 mm.). The infrared spectra of the two isomers are described.[6]

6. The procedure is an adaptation of that of Winstein.[6]

7. *endo*-2-Norcamphanol and *exo*-2-norcamphanol melt[6] at 152–153° and 127.8–128.5°, respectively.

8. The enrichment procedure is based on the stereochemical equilibration method of Doering and Aschner[7] for alcohols.

It has been demonstrated that isomerization of a bicyclic Diels-Alder adduct of *endo*-stereochemistry to the *exo*-isomer can occur without dissociation into kinetically free addends.[8] When *endo-cis*-bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic-C_2^{14} anhydride, (*cis*-3,6-*endo*-methylene-1,2,3,6-tetrahydrophthalic-C_2^{14} anhydride), prepared by adding maleic-C_2^{14}

anhydride to cyclopentadiene, was heated with an equimolar amount of maleic anhydride in boiling decahydronaphthalene, there followed a rapid exchange of maleic anhydride with the *endo*-adduct and a slower conversion to the *exo*-isomer,[8] which was determined by isotope dilution technique. Exchange of maleic anhydride with the *exo*-adduct was small at short reaction times. This isomerization does not occur with the dihydro-form and appears to be not catalyzed by bases.

9. The mixture is analyzed by heating a 7% solution of the *p*-bromobenzenesulfonate mixture in 75% acetone- 25% water (v/v) for 30 minutes at 45°. Titration of the liberated *p*-bromobenzenesulfonic acid gives the *exo*- content of the mixture. The unreacted *endo*-2-norcamphanyl-2,3-C_2^{14} *p*-bromobenzenesulfonate is recovered almost quantitatively by extracting the neutral solution with ether and precipitating the ester from the dried, concentrated ether solution with pentane. The filtrate is evaporated, and the residue is sublimed to yield *exo*-2-norcamphanol-2,3-C_2^{14}; m.p. 127–128°, yield 60–65% based on reacted *exo*-ester. The carbanilate melts at 145–146°.

10. The procedure is adapted from that of Tipson[9] for preparing tosylates.

11. *exo*-2-Norcamphanyl-2,3-C_2^{14} *p*-bromobenzenesulfonate, prepared from pure *exo*-2-norcamphanol-2,3-C_2^{14}, melts at 60°. *endo*-2-Norcamphanyl-2,3-C_2^{14} *p*-bromobenzenesulfonate, prepared from pure *endo*-2-norcamphanol-2,3-C_2^{14}, melts at 62–63°.

12. This preparation is incidental to a study of the rearrangement mechanism of the solvolysis reaction; many literature references are given by Winstein[6] and by Roberts. Solvolysis of the *exo*- and *endo*-2-norcamphanyl-2,3-C_2^{14} *p*-bromobenzenesulfonates yields exclusively substitution products (acetate, formate, carbinol) with the *exo*- configuration, since the reaction rate of the *exo*-compounds is up to 350 times greater than that of the *endo*-isomers.

13. Any *endo*-sulfonate ester present may be precipitated by dilution with pentane. In the case of the less reactive *endo*-esters, the solvolysis reaction is conducted under reflux for 20 hours (yield 66%).

14. There is circumstantial evidence that there is no isotope at C-4. The equilibrium extent of rearrangement of these esters appears to be 67–70%.

15. The acetate (g) is cleaved in the manner described above.

16. A dark, intractable syrup results if the evaporation is not carried out with care.

17. Directions are given by Roberts for decarboxylating the acid with hydrazoic acid, forming barium carbonate-C^{14} (70–80% yield) and C^{14}-*cis*-1,3-cyclopentanediamine dihydrochloride (70–90% yield), (i), possessing 39% and 60% of the initial activity, respectively. The amine may be assayed as the complex,[10] C^{14}-*cis*-1,3-cyclopentanediamine stannous

chloride dihydrate, m.p. 145–146°; or preferably, as *cis-N,N'*-(C^{14}-1,3-cyclopentylene)bisbenzamide, m.p. 213–215°.

The diamine dihydrochloride is oxidized with permanganate to C^{14}-succinic acid, (k), (m.p. 188–189°), possessing 38% of the activity of (h). The acid may be assayed as *p*-bromophenacyl C^{14}-succinate, m.p. 213–214°.[11]

The C^{14}-succinic acid is converted to C^{14}-ethylenediamine dihydrobromide by the Curtius degradation (see succinic-2-C^{14} acid, Note 12). The activities of the diamine and of the carbon dioxide formed are 15% and 23%, respectively, of the activity of (h).

The difference in activity between (i) and (k) represents the activity of C-7. The activity of (l) gives that of C-5,6. The activity of (m) is derived from C-1,4.

C. Other Preparations

In the same manner, by solvolysis of *exo*- or *endo*-2-norcamphanyl-2,3-C_2^{14} *p*-bromobenzenesulfonate in 98–100% formic acid, is prepared C_2^{14}-*exo*-2-norcamphanyl formate; yield 66–75%, b.p. 79–80° (25 mm.).

By similar methods (also see Winstein[12] and Roberts[13]) from dehydronorbornyl-2,3-C_2^{14} acetate are prepared: dehydronorborneol-2,3-C_2^{14} (yield 82%), *exo*-dehydronorborneol-2,3-C_2^{14} (yield 76%, purity 47%), dehydronorbornyl-2,3-C_2^{14} *p*-bromobenzenesulfonate and *endo*-2-norcamphanyl-2,3-C_2^{14} *p*-bromobenzenesulfonate (recovery 51%, m.p. 88–89°). *endo*-Dehydronorborneol, *exo*-dehydronorborneol and 3-hydroxynortricyclene melt[13] at 109.4–110.8°, 97.5–99.2° and 107.8–108.8°, respectively. The corresponding carbanilates melt[13] at 129.4–129.9°, 149.2–151.8° and 146–147.5°. The infrared spectra are described. *endo*-Dehydronorbornyl *p*-bromobenzenesulfonate and crude *exo*-dehydronorbornyl *p*-bromobenzenesulfonate melt at 87.4–89.0° and 78.4–79.8°, respectively.

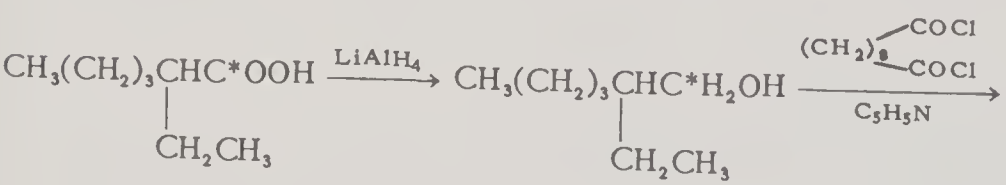
Solvolysis of *exo*- and *endo*-dehydronorbornyl-2,3-C_2^{14} *p*-bromobenzenesulfonates in acetic acid (or formic acid solution) yields 4–17% C_2^{14}-*exo*-dehydronorbornyl acetate (or formate) having 30–48% of the isotope rearranged to positions other than C-2,3, on the basis of degradation data. The yield is based upon the subsequent absorption of hydrogen by the mixture; the chief product is C_2^{14}-3-nortricyclyl acetate (or formate). Cleavage of the crude product yields a mixture of C_2^{14}-tricyclo-[2.2.1.$0^{2,6}$]heptan-3-ol, (C_2^{14}-3-hydroxynortricyclene) and C_2^{14}-*exo*-dehydronorborneol, the latter being converted to C_2^{14}-*exo*-norborneol by hydrogenation.

The similar preparation of 2-phenyl-2-(*p*-tolyl)ethyl-1-C^{14} *p*-toluenesulfonate has been described by Burr,[14] m.p. 108° from ethyl acetate-hexane. Solvolysis for 16 hours at 100° in dry glacial acetic acid gave

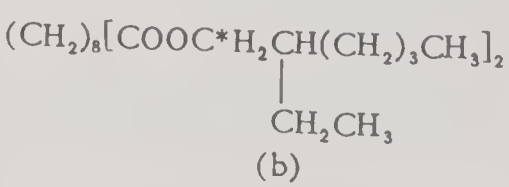
4-methylstilbene-α,α′-$C^{14}_{1/2}$, m.p. 110° from methanol. Degradation with alkaline permanganate gave terephthalic-C^{14}_1 acid having 71.2% of the molar specific activity of the starting material. Reaction of the ester with sodium methoxide in methyl cellosolve gave 4-methylstilbene-α′-C^{14} which contained less than 1.9% of the activity at C-α. A kinetic study is described.

[1]R. F. Nystrom and W. G. Brown, J. Am. Chem. Soc., *69*, 1197 (1947).
[2]G. O. Morrison and T. P. G. Shaw, Trans. Electrochem. Soc., *63*, 425 (1933).
[3]B. S. Groth and S. B. H. Johanson, U.S. 2,376,964; Chem. Abstracts, *39*, 3303 (1945).
[4]K. Alder and H. F. Rickert, Ann., *543*, 1 (1939); *Organic Reactions*, Vol. IV, Wiley, New York, 1948, Chapter 2.
[5]J. D. Roberts, E. R. Trumbull, Jr., W. Bennett and R. Armstrong, J. Am. Chem. Soc., *72*, 3116 (1950).
[6]S. Winstein and D. Trifan, J. Am. Chem. Soc., *71*, 2953 (1949); *74*, 1147, 1154 (1952).
[7]W. v. E. Doering and T. E. Aschner, J. Am. Chem. Soc., *71*, 838 (1949).
[8]J. A. Berson and R. D. Reynolds, J. Am. Chem. Soc., *77*, 4434 (1955).
[9]R. S. Tipson, J. Org. Chem., *9*, 235 (1944).
[10]O. Diels, J. H. Blom and W. Koll, Ann., *443*, 242 (1925).
[11]W. L. Judefind and E. E. Reid, J. Am. Chem. Soc., *42*, 1043 (1920).
[12]S. Winstein, H. M. Walborsky and K. Schreiber, J. Am. Chem. Soc., *72*, 5795 (1950).
[13]J. D. Roberts, E. R. Trumbull, Jr., W. Bennett and R. Armstrong, J. Am. Chem. Soc., *72*, 3116 (1950).
[14]J. G. Burr, Jr., J. Am. Chem. Soc., *75*, 5008 (1953); J. G. Burr, Jr., Chem. and Ind., *1954*, 850.

BIS(2-ETHYLHEXYL-1-C^{14}) DECANEDIOATE



(a)



(b)

J. L. Kalinsky and A. Weinstein, J. Am. Chem. Soc., *76*, 3730 (1954).

A. Procedure

(a) *2-Ethylhexanol-1-C^{14}*. To a dry ether solution of 1.96 g. (13.6 mmoles) of 2-ethylhexanoic-1-C^{14} acid is added slowly 25 ml. of 1 *M* lithium aluminum hydride in ether, care being taken to exclude carbon dioxide and moisture. The mixture is decomposed with dilute sulfuric

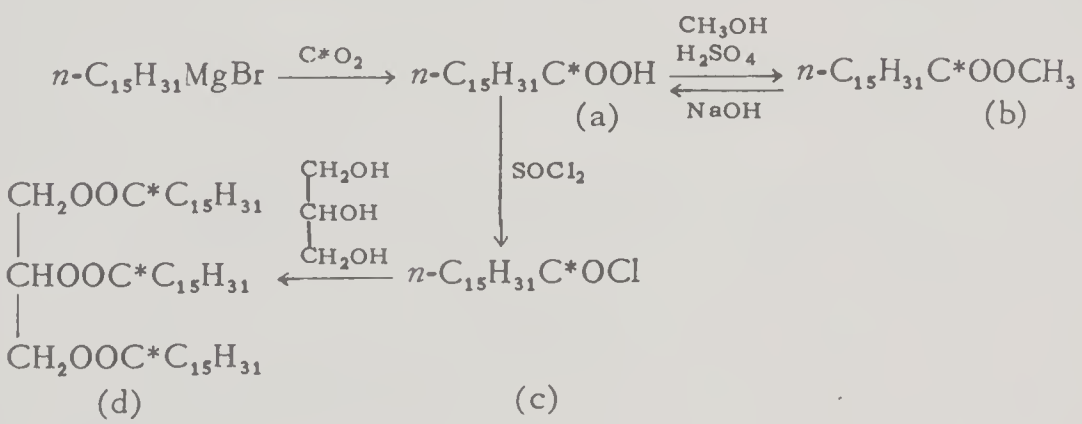
acid, then washed with sodium bicarbonate and water. The solution is dried and concentrated by flash distillation of the solvent, and the residue is diluted with 10.0 g. of carrier. The product is distilled, the system being scavanged with another 10.0-g. portion of carrier; yield 21.2 g. (96%), b.p. 182°, n_D 1.4273.

(b) *Bis (2-ethylhexyl-1-C^{14}) Decanedioate.* To a solution of 7.4 g. (57 mmoles) of 2-ethylhexanol-1-C^{14} and 4.5 g. (57 mmoles) of pyridine in 15 ml. of anhydrous benzene is added slowly 6.8 g. (29 mmoles) of sebacoyl chloride in 15 ml. of benzene. The mixture is washed with water and sodium bicarbonate, dried and concentrated by flask distillation, and the residue is distilled; yield 9.4 g. (77%), b.p. 180° (0.3 mm.), n_D 1.4490.

B. Other Preparations

In a similar manner is prepared bis(2-ethylhexyl-1-C^{14}) phthalate; yield 72%, b.p. 168° (0.3 mm.), n_D 1.4825. In this case, however, the pyridine is added to the mixture of alcohol and acid chloride to minimize the formation of colored condensation products. The crude ester may be steam-distilled, in lieu of washing with *N* sodium hydroxide, to remove the phthalic anhydride.

GLYCERYL TRIS(HEXADECANOATE-1-C^{14})
(C_3^{14}-Tripalmitin)



W. G. Dauben, J. Am. Chem. Soc., 70, 1376 (1948).

A. Procedure

(a) *Hexadecanoic-1-C^{14} Acid, (Palmitic-C^{14} Acid).* A Grignard solution 110 ml.) containing 48.4 mmoles of pentadecylmagnesium bromide (Note 1) is carbonated with the carbon-C^{14} dioxide from 9.1 g. (46 mmoles) of barium carbonate-C^{14} (see benzoic-C^{14} acid). The crude product is isolated by extraction with dilute sodium hydroxide, followed by acidification, extraction with ether and evaporation (Note 2).

(b) *Methyl Hexadecanoate-1-C^{14}.* The crude acid is refluxed for 1 hour with 48.6 ml. of methanol and 5 ml. of sulfuric acid; the solution is diluted with 200 ml. of water, and the product is extracted into hexane. The extract is washed with water, dried over magnesium sulfate and concentrated at reduced pressure, and the product is isolated by fractional distillation. The yield is 10.0 g. (80.4% based on carbon dioxide), n_D^{25} 1.4386, b.p. 132–133° (0.3 mm.) (Note 2).

(c) *Hexadecanoyl-1-C^{14} Chloride, (Palmitoyl-C^{14} Chloride).* A mixture of 8.3 g. of hexadecanoic-1-C^{14} acid and 10 ml. of purified thionyl chloride is refluxed about 2 hours, then the excess thionyl chloride is removed at reduced pressure (Note 3). The residue is treated with 25 ml. of anhydrous benzene, and evaporated under reduced pressure (three times) to remove the last traces of the reagent.

(d) *Glyceryl Tris(hexadecanoate-1-C^{14}), (Glyceryl Palmitate-C^{14}).* (Note 4). A solution of the hexadecanoyl-1-C^{14} chloride in 25 ml. of dry chloroform (Note 5) is added slowly to a stirred, cooled mixture of 0.975 g. (106 mmoles) of redistilled glycerol, 8 ml. of anhydrous pyridine and 25 ml. of chloroform. The solution is stored at room temperature for 3 days (Note 6), then refluxed for 2 hours. The solvent is evaporated, and the residue is treated with 100 ml. of 0.5 *N* sulfuric acid. The syrup is extracted into 500 ml. of ether, and the solution is washed with 100 ml. of 0.5 *N* sulfuric acid, followed by 200 ml. of 5% sodium carbonate solution (Note 7). The solution is dried over magnesium sulfate and concentrated at reduced pressure, and the residue is recrystallized twice from acetone, decolorizing with Norit. The yield is 6.15 g. (75.5% based on palmitic acid), m.p. 61–62° (sinters 59°).

B. Notes

1. Directions are given by Dauben for preparing 1-pentadecanol from paraformaldehyde and tetradecylmagnesium bromide, or by hydrogenation of ethyl pentadecanoate over copper chromite. The halide is prepared with anhydrous hydrogen bromide. The Grignard reagent is prepared under nitrogen from 1.6 g. of magnesium turnings and 15.9 g. (54.7 mmoles) of 1-bromopentadecane in 110 ml. of absolute ether. The solution is assayed by titration of an aliquot.

The bromide is prepared in high yield by degradation[1] of silver palmitate with bromine.

2. The acid is purified most effectively through fractional distillation of the ester. The ester is refluxed overnight with 2.3 g. of potassium hydroxide, 35 ml. of methanol and 2 ml. of water. The alcohol is distilled, and the residue is acidified with hydrochloric acid and heated for 1 hour on a steam-bath. The mixture is extracted with ether, washed with water and dried over magnesium sulfate, and the solvent is evap-

orated. The crude acid is recrystallized from 40 ml. of 10% aqueous acetone; yield 8.55 g. (90.1%), 72.4% based on carbon dioxide, m.p. 60–61°.

The decarboxylation of palmitic-C^{14} acid and higher fatty acids, for tracer work, has been described by Blomstrand[2] (60–90% yield).

3. The vacuum of a water pump is satisfactory, suitable precautions being taken to ensure anhydrous conditions.

4. The procedure is adapted from the method of Stephenson.[3]

5. The chloroform is freed from alcohol by shaking with concentrated sulfuric acid, washing with water, drying with calcium chloride and phosphorus pentoxide, and distilling. Storage at low temperature minimizes the formation of phosgene.

6. 1,3-Dipalmitin, m.p. 73.0–73.5°, may be prepared[4] in 67% yield, if the reaction is not allowed to proceed longer than 2 hours.

7. Emulsions may be broken by adding sodium chloride.

C. Other Preparations

The preparation of sodium hexadecanoate-1-C^{14} by a similar procedure has been reported by Bergström,[5] Meader,[6] Jedeikin,[7] and by Geyer[8] (86%); potassium hexadecanoate-1-C^{13} by Weinhouse.[9] The acid has been purified[6] through the silver salt, which was decomposed with sodium sulfide.

The glyceryl esters of hexadecanoic-1-C^{14}, -6-C^{14}, and -11-C^{14} acids have been prepared by Weinman,[10] by a modification of the method of Feuge.[11] Yields are excellent, apparently, as the method is used extensively.

The glyceryl esters of octadecanoic-1-C^{14} and -6-C^{14} acids also have been prepared in like manner by Weinman.[11,12]

[1] A. Hunsdiecker and C. Hunsdiecker, Ber., *75*, 291 (1942).

[2] R. Blomstrand, Acta Chem. Scand., *8*, 1847 (1954).

[3] M. Stephenson, Biochem. J., *7*, 429 (1913).

[4] W. G. Rose, J. Am. Chem. Soc., *69*, 1384 (1947).

[5] S. Bergström, B. Borgström and M. Röttenberg, Acta Physiol. Scand., *25*, 120 (1952).

[6] A. L. Meader, Jr., and B. A. Fries, Ind. Eng. Chem., *44*, 1636 (1951).

[7] L. A. Jedeikin and S. Weinhouse, Arch. Biochem. Biophys, *50*, 134 (1954).

[8] R. P. Geyer, W. R. Waddell, J. Pendergast and G. S. Yee, J. Biol. Chem., *190*, 437 (1951).

[9] S. Weinhouse, R. H. Millington and M. E. Volk, J. Biol. Chem., *185*, 191 (1950).

[10] E. O. Weinman, I. L. Chaikoff, W. G. Dauben, M. Gee and C. Entenman, J. Biol. Chem., *184*, 735 (1950).

[11] R. O. Feuge, E. A. Kraemer and A. E. Bailey, Oil and Soap, *22*, 202 (1945); Chem. Abstracts, *39*, 4504 (1945).

[12] E. O. Weinman, I. L. Chaikoff, B. P. Stevens and W. G. Dauben, J. Biol. Chem., *191*, 523 (1951).

METHYL LINOLEATE-1-C^{14}

$$CH_3(CH_2)_4\underset{H}{C}=\underset{H}{C}CH_2\underset{H}{C}=\underset{H}{C}(CH_2)_6CH_2Br \xrightarrow[2.\ C^*O_2]{1.\ Mg}$$

$$RC^*OOH \xrightarrow{CH_2N_2} RC^*OOCH_3$$
(a) (b)

D. R. Howton, R. H. Davis and J. C. Nevenzel, J. Am. Chem. Soc., 74, 1109 (1952); 76, 4970 (1954); D. R. Howton and J. C. Nevenzel, Atomic Energy Commission Report, UCLA-257; Nuc. Sci. Abstracts, 7, 4360 (1953).

A. Procedure (Note 1)

(a) *Linoleic-1-C^{14} Acid*, (cis,cis-*9,12-Octadecadienoic-1-C^{14} Acid*). To 0.0807 g. (3.32 mmoles) of magnesium turnings under nitrogen is added 3 ml. of a solution containing 0.9838 g. (3.12 mmoles) of 1-bromo-8,11-heptadecadiene (Note 2) in 20 ml. of absolute ether (Note 3), and the mixture is stirred for 1.5 hours. The bulk of the halide solution then is added to the grey-black mixture during 3.75 hours, stirring is continued for an additional 1.75 hours, and the mixture is stored overnight at room temperature. The Grignard reagent is assayed (Note 4), then frozen in liquid nitrogen, and the carbon-C^{14} dioxide (26.3% excess) liberated from 3.23 mmoles of barium carbonate-C^{14} is distilled in. The mixture is stirred for 80 minutes at -20° and stored overnight at room temperature; then the system is swept with nitrogen to recover unreacted carbon-C^{14} dioxide (1.39 mmoles, 43%). The neutral and acidic products are separated by extraction. Titration shows that the yield of product is 40% based on the Grignard reagent, 31% based on the bromide, and 29.3% based on unrecovered barium carbonate (Note 5).

(b) *Methyl Linoleate-1-C^{14}*. The crude acid is treated with about 10.7 mmoles of diazomethane in ether, and the solution is set aside for about 6 hours, then evaporated under vacuum. The residue is passed with petroleum ether (b.p. 60–70°) through a column of alumina-Celite (Note 6), and the product is collected in about 200 ml. of eluate (Note 7). Evaporation yields 0.250 g. of product, 90% based on the crude acid. The over-all radiochemical yield is 44%.

B. Notes

1. The method appears to be general for the decarboxylation and reconstitution of naturally occurring unsaturated fatty acids labeled in the carboxyl group. Contact of the products with air is avoided at all times. A total synthesis of linoleic acid, preliminary to labeling with

isotopic carbon in other specific positions, has been reported by Walborsky.[1]

2. Detailed directions are given by Howton for preparing the starting halide. The carboxyl group of linoleic acid, isolated from natural sources as the readily purified 9,10,12,13-tetrabromoöctadecanoic acid[2] (thus preserving the sensitive *cis,cis*-1,4-diene grouping), is removed by degrading the silver salt with dry bromine in boiling carbon tetrachloride; also see oleic-1-C^{13} acid. The desired 1,8,9,11,12-pentabromoheptadecane is chromatographically resolved from the reaction mixture (yield 70–71%), then debrominated in boiling alcohol-benzene with zinc (yield 88.5%), regenerating the original synthetically-imposing diene grouping without affecting the terminal bromine; b.p. 118–122° (0.1 mm.), n_D^{25} 1.4782.

3. The ether is freshly distilled from propylmagnesium bromide. Despite the sluggishness with which this long chain halide reacts with magnesium, good yields are obtained only at high dilution, or when the halide is added slowly. The Wurtz-type coupling becomes an increasingly important side reaction with the higher aliphatic halides.

4. An aliquot (0.25 ml./10.0 ± 0.3 ml.) is removed by pipette and titrated with acid[3] (methyl orange). The indicated yield of 78% is understandably high in the light of later studies by Gilman[4] in developing the improved titration procedure using phenolphthalein.

5. Analysis of the neutral by-products (0.365 g.) shows that they contain only 0.12% of the original radioactivity, thus ruling out ketonic and alcoholic components. The acid neutralization equivalent of reconstituted linoleic acid is 298 (calc. 280), indicating the presence of about 6% neutral material. The ultraviolet absorption spectrum indicates the presence of 3.9% conjugated diene and 0.7% conjugated triene. The infrared absorption at 10.3 μ indicates the presence of about 15% *trans*-isomer. An aliquot converted to 9,10,12,13-tetrabromoöctadecanoic-1-C^{14} acid, m.p. 114–115°, showed no depression by admixture with an authentic sample.[2]

6. The 2 × 10-cm. column consists of equal weights of the adsorbents, pre-washed with the solvent.

7. Autoöxidation and saponification products are retained on the column.

8. The primary bromine of 1,8,9,11,12-pentabromoheptadecane (see Note 2) is removed by hydrolysis with sodium trifluoroacetate in good yield. The resulting carbinol is debrominated with zinc and esterified with *p*-toluenesulfonyl chloride.

C. Other Preparations

The preparation of linoleic-1-C^{14} acid by the nitrile synthesis has been reported by Röttenberg;[5] see oleic-1-C^{13} acid. The appropriate unsaturated

tosylate (Note 8) was reacted with potassium cyanide-C^{14}, and the resulting *cis,cis*-9,12-octadecadienenitrile-1-C^{14} was hydrolyzed with acid. The infrared spectrum indicated that the product contained 7% of an isomer with a conjugated double bond system, presumably formed under the alkaline conditions of the condensation.

[1]H. M. Walborsky, R. H. Davis and D. R. Howton, J. Am. Chem. Soc., *73*, 2590 (1951); W. J. Gensler and G. R. Thomas, *ibid*, *73*, 4601 (1951).

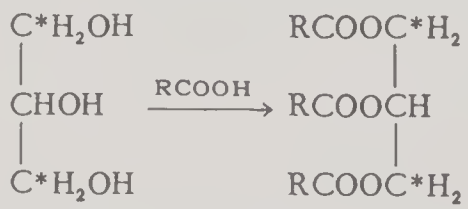
[2]*Organic Syntheses*, Vol. 22, Wiley, New York, 1942, p. 75.

[3]H. Gilman, P. D. Wilkinson, W. P. Fishel and C. H. Meyers, J. Am. Chem. Soc., *45*, 150 (1923).

[4]H. Gilman, E. A. Zoellner and J. B. Dickey, *ibid.*, *51*, 1576 (1929).

[5]M. Röttenberg, Angew. Chem., *66*, 79 (1954); C. R. Noller and M. D. Girvin, J. Am. Chem. Soc., *59*, 606 (1937).

GLYCERYL-1,3-C_2^{14} 10,12-OCTADECADIENOATE
(C_2^{14}-Conjugated Trilinolein)



[R = $CH_3(CH_2)_4CH{=}CHCH{=}CH(CH_2)_8$ —]

R. Reiser, M. J. Bryson, M. J. Carr and K. A. Kuiken, J. Biol. Chem., *194*, 131 (1952).

A. Procedure (Note 1)

A mixture of dried conjugated linoleic acid (Note 2), 90% of the equivalent weight of 1,2,3-propanetriol-1,3-C_2^{14} and 2-naphthalenesulfonic acid catalyst is refluxed at 150–160° in dry xylene until there is no further evolution of water, which is collected in a trap. The excess fatty acid is removed by shaking with Amberlite 400.

B. Notes

1. The procedure is a modification of the method of Reiser and Bryson[1] and is general for preparing glyceryl esters.

2. It has been shown that conjugated linoleic acid may be used as a tracer in biological systems. It is prepared according to the directions of Reiser:[1] linoleic acid is isolated[2] from natural sources and esterified with diazomethane, the ester is fractionated by the urea-complex method,[3] the diene group is isomerized[4] by heat-bodying, the ester is saponified,

and the conjugated acid is assayed by the spectophotometric procedure of Brice.[5]

[1]R. Reiser and M. J. Bryson, J. Biol. Chem., *189*, 87 (1951).
[2]*Organic Syntheses*, Vol. 22, Wiley, New York, 1942, p. 75.
[3]H. Schlenk and R. T. Holman, J. Am. Chem. Soc., *72*, 5001 (1950).
[4]T. F. Bradley and D. Richardson, Ind. Eng. Chem., *32*, 963 (1940).
[5]B. A. Brice, M. L. Swain, B. B. Schaeffer and W. C. Ault, Oil and Soap, *22*, 219 (1945).

ETHYL MALONATE-2-C^{14}

$$C^*H_2(COONa)_2 \xrightarrow{AgNO_3} \underset{(a)}{C^*H_2(COOAg)_2} \xrightarrow{C_2H_5I} \underset{(b)}{C^*H_2(COOC_2H_5)_2}$$

D. Chin and P. Adams, Atomic Energy Commission Report, UCRL-2394; Nuc. Sci. Abstracts, *8*, 1057 (1954).

A. Procedure

(a) *Silver Malonate-2-C^{14}*. To a stirred, clear, aqueous solution of sodium malonate-2-C^{14}, prepared from 20–25 mmoles of sodium acetate-2-C^{14}, is added slowly a solution of 8.5 g. (50 mmoles) of silver nitrate in 25 ml. of water (Note 1). The copious gray precipitate is collected (Note 2), washed with a small amount of water and dried under vacuum.

(b) *Ethyl Malonate-2-C^{14}*. A stirred mixture of 30 ml. of ethyl iodide and the silver malonate-2-C^{14} is refluxed for 3 hours, then stirred overnight at room temperature. The product and excess ethyl iodide are filtered into a 60-ml. dropping funnel, and the silver residue is washed with 20 ml. of ether in 4–5 portions. The volatile solvents are removed from the combined solutions by flash distillation at atmospheric pressure from a 30-ml., two-necked pear-shaped flask, followed by evacuation at 30 mm. for 30 minutes at room temperature. The residue is distilled at 30 mm., and the crude product is collected at 97–103° (Note 3); yield 81–85% based on sodium acetate. Redistillation gives a yield of 60–70% based on sodium acetate, b.p. 105–106° (30–31 mm.), n_D^{23} 1.4130. The molar specific activity is 94% of that of the starting material.

B. Notes

1. The apparatus consists of a 200-ml. round-bottomed flask equipped with a magnetic stirrer and a side arm fitted with a sintered glass filter, which is sealed with a glass cap over the stem.

2. The filtrate is tested with silver nitrate.

3. The distillate is collected in a similar distillation flask to avoid transfer.

C. Other Preparations

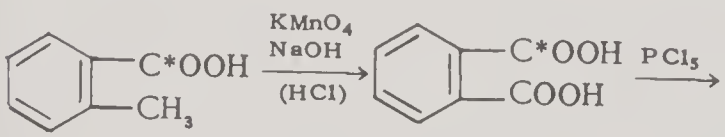
Gidez and Karnovsky[1] have prepared ethyl malonate-2-C^{14} (73% yield) by esterification of the calcium salt with absolute ethanol and dry hydrogen chloride, but Chin and Adams have reported yields of only 30% by this method.

Gianetto[2] has prepared ethyl malonate-2-C^{14} (30–35% yield based on carbon dioxide) by treating sodium cyanoacetate-2-C^{14} with alcohol and sulfuric acid in the presence of benzene.[3]

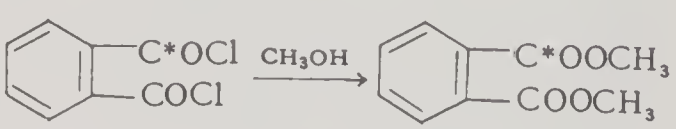
Ropp[4] has prepared ethyl malonate-2-C^{14} by condensing ethyl acetate-2-C^{14} with ethyl oxalate, forming ethyl oxalacetate-3-C^{14} (51%) which was catalytically decarbonylated in the vapor-phase at 350° (71%). The over-all yield was 28.5% based on unrecovered ethyl acetate and corrected for a product purity of 58%, which was determined by isotope dilution and conversion of the ester to the free acid.

[1]L. I. Gidez and M. L. Karnovsky, J. Am. Chem. Soc., *74*, 2413 (1952).
[2]R. Gianetto and L. P. Bouthillier, Can. J. Biochem. and Physiol., *32*, 154 (1954).
[3]C. F. Christie, U. S. 2,459,144; Chem. Abstracts, *43*, P 3031 (1949).
[4]G. A. Ropp, J. Am. Chem. Soc., *72*, 4459 (1950); C. J. Collins and G. Ropp, Nucleonics, *7* (3), 58 (1950).

METHYL PHTHALATE-C_1^{14}



(a)



(b)

D. Y. Barker and J. E. Christian, J. Am. Pharm. Assoc., Sci. Ed., *44*, 105 (1955).

A. Procedure

(a) *Phthalic-C_1^{14} Acid.* A stirred mixture of the *o*-toluic-C^{14} acid prepared from 10 mmoles of barium carbonate-C^{14}, 60 ml. of 1 *N* sodium hydroxide, 3.48 g. (10% excess) of potassium permanganate and 80 ml. of water is heated on a steam-bath for 10 hours. The excess oxidant is reduced with 5 ml. of alcohol, and the mixture is cooled, acidified (litmus) with hydrochloric acid and heated again for 30 minutes at the

steam-bath. The precipitated manganese dioxide is dissolved by the addition of saturated sodium bisulfite solution (3 ml.), and the clear solution is concentrated to 20 ml. under a warm air stream. The solution is cooled with an ice-bath, and the crystals are collected on a filter, the mother liquor being scavanged with 0.2 g. of carrier. The crude product is recrystallized from water; yield 1.6 g., 84.4% based on barium carbonate (Note 1).

(b) *Methyl Phthalate-C_1^{14}*. The phthalic-C^{14} acid is mixed intimately with 6.32 g. (30.3 mmoles) of phosphorus pentachloride in a 125-ml. Erlenmeyer flask, while being heated over a small flame. The clear solution of phthaloyl-C_1^{14} chloride is cooled with an ice-bath, and the flask is fitted with a 25-cm. air condenser, through which is added 5 ml. of absolute methanol in small portions. The solution is mixed with 2 g. of sodium carbonate in 5 ml. of water, and the product is extracted with three 10-ml. portions of ether. The extract is concentrated, dried over calcium chloride (48 hours) and transferred into a 10-ml. distilling flask with 0.5-ml. portions of methanol. The product is collected by distillation at 275–279°; yield 1.79 g., 80.4% based on barium carbonate. Paper chromatography (Note 2) and radioautography indicate the presence of only one isotopic compound.

B. Notes

1. Phthalic acid melts at about 230° when rapidly heated, decomposing into the anhydride. The water solubility is 1 g. in 160 ml. of water.

2. Directions are given for developing the chromatogram with alcohol-water-formic acid (49 : 49 : 2); R_f 0.88, Whatman paper No. 1 is impregnated with acetone-formamide (70 : 30) for 12 hours and dried at room temperature for 24 hours before use. The ester is located chemically with Dragendorff's reagent, producing a yellow-brown spot. The reagent[1] consists of a suspension of 1.5 g. of bismuth subnitrate in 20 cc. of hot water to which is then added 7 g. of potassium iodide and 20 drops of dilute hydrochloric acid.

Methyl phthalate d_{25}^{25} 1.189, n_D^{21} 1.5154.

C. Other Preparations

The preparation of phthalic-C_1^{14} acid by essentially the described procedure has been outlined by Stevens[2] and Hadler.[3]

[1] *Hackh's Chemical Dictionary*, Blakiston, New York, 1953.
[2] W. H. Stevens and D. A. Holland, Science, *112*, 718 (1950).
[3] H. I. Hadler, J. Am. Chem. Soc., 77, 1052 (1955).

ETHYL CYANO-C^{14}-ACETATE

$$ClCH_2COOH \xrightarrow{NaC^*N} NC^*CH_2COOH \xrightarrow[H_2SO_4]{C_2H_5OH} NC^*CH_2COOC_2H_5$$

(a) (b)

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A. Procedure (Note 1)

(a) *Cyano-C^{14}-acetic Acid.* A solution of 16 g. (170 mmoles) of chloroacetic acid in 25 ml. of water at 50° is made alkaline slowly with 9.6 g. (90 mmoles) of sodium carbonate. To the stirred solution at 0° is added slowly 8.80 g. (180 mmoles) of sodium cyanide-C^{14} in 30 ml. of ice-cold water (Note 2). The solution is stored at room temperature for 30 minutes, heated to boiling for 5 minutes, then cooled in an ice-bath for 30 minutes and slowly acidified with 20 ml. of concentrated hydrochloric acid. The solution is evaporated at 60° under reduced pressure; then the residue is taken up in 95% ethanol, and the sodium chloride is removed by centrifuging and washed with several portions of alcohol. The combined solution and washings are evaporated at 45° under reduced pressure.

(b) *Ethyl Cyano-C^{14}-acetate* (Note 3). The crude cyano-C^{14}-acetic acid is refluxed for 3 hours with 30 ml. of absolute ethanol and 4 ml. of concentrated sulfuric acid. The mixture is evaporated under reduced pressure, then refluxed for 2 hours with additional absolute alcohol and again evaporated. The esterification procedure is repeated once more; then, with ice-bath cooling, the crude ester is treated with an excess of aqueous sodium carbonate solution and extracted into ether. The combined ether solutions are dried overnight with sodium sulfate and concentrated at 50° under reduced pressure; yield 17.45 g., 86% based on cyanide (Note 4).

B. Notes

1. The procedure is a modification of the procedure of Inglis;[1] also see Weiner.[2] A review of the preparation of nitriles is presented by Mowry.[3]

2. Preventing an excessive rise in temperature increases the yield.

3. For another preparation see adenine-4,6-$C^{14}_{1/2}$ sulfate.

4. The product is sufficiently pure for many purposes. A distilled sample exhibits the physical properties of an authentic sample, n_D^{25} 1.4151.

C. Other Preparations

Ethyl cyano-C^{13}-acetate has been prepared[4] by the method of Inglis,[1] and the preparation of ethyl cyano-C^{14}-acetate has been described[5] (yield 70%).

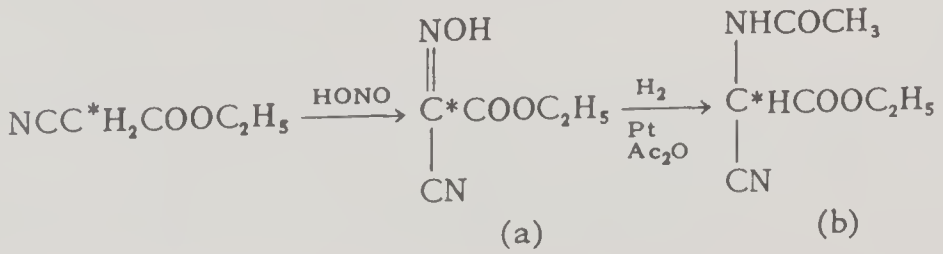
The preparation of ethyl cyano-C^{14}-acetate by condensation of sodium cyanide-C^{14} and ethyl chloroacetate in alcohol solution has been described by Bouthillier.[6]

Cyano-C^{14}-acetic acid has been prepared in essentially the described manner by Bennett,[7] Yankwich (see malonic-1-C^{14} acid), Cavalieri,[8] Fritzson (see β-alanine-3-C^{14}) and Freudenberg (see α-cyano-C^{14}-ferulic acid). Cyanoacetic-C_2^{14} acid has been prepared in a similar fashion by Gal.[9] Fields[10] and Gianetto[11] have prepared cyanoacetic-2-C^{14} acid from the bromoacetate-2-C^{14}, and Bentley[12] has prepared cyanoacetic-1-C^{14} acid from bromoacetic-1-C^{14} acid. The esterification has been effected with diazoethane,[7] diazomethane[8] (42% yield based on cyanide), methyl sulfate,[9] and with absolute ethanol-sulfuric acid.[10]

The preparation of methyl cyanoacetate-1-C^{14} by condensation of methyl bromoacetate-1-C^{14} with sodium cyanide has been reported by Brandenberger.[13]

[1] *Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 254.
[2] *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 376.
[3] D. T. Mowry, Chem. Revs., *42*, 189 (1948).
[4] J. E. Seegmiller, L. Laster and D. Stetten, Jr., J. Biol. Chem., *216*, 653 (1955).
[5] C. E. Dalgliesh and A. Neuberger, J. Chem. Soc., *1954*, 3407
[6] L. P. Bouthillier and A. D'Iorio, Rev. can. biol., *9*, 382 (1950); O. C. Stephens, J. Soc. Chem. Ind., *43T*, 313 (1924).
[7] E. L. Bennett, J. Am. Chem. Soc., *74*, 2420 (1952).
[8] L. F. Cavalieri, J. F. Tinker and A. Bendich, J. Am. Chem. Soc., *71*, 533 (1949).
[9] E. M. Gal and A. T. Shulgin, J. Am. Chem. Soc., *73*, 2938 (1951).
[10] M. Fields, D. E. Walz and S. Rothchild, J. Am. Chem. Soc., *73*, 1000 (1951).
[11] R. Gianetto and L. P. Bouthillier, Can. J. Biochem. and Physiol., *32*, 154 (1954); H. A. Scarborough, Proc. Chem. Soc., *30*, 306 (1944).
[12] R. Bentley and A. Neuberger, Biochem. J., *52*, 694 (1952).
[13] H. Brandenberger, Biochem. Biophys. Acta, *15*, 108 (1954); W. A. Noyes, J. Am. Chem. Soc., *26*, 1545 (1904).

ETHYL ACETAMIDOCYANOACETATE-2-C^{14}



M. Fields, D. E. Walz and S. Rothchild, J. Am. Chem. Soc., *73*, 1000 (1951).

A. Procedure

(a) *Ethyl Cyanohydroxyiminoacetate-2-C^{14}.* The nitrosation of ethyl cyanoacetate-2-C^{14} is performed by the method of Snyder and Smith.[1] To a stirred solution of ethyl cyanoacetate-2-C^{14} in glacial acetic acid, cooled in an ice-bath, is added slowly a three-fold molar amount of sodium nitrite in water, while the temperature is kept below 20°. The mixture is stirred for several hours, then acidified with dilute hydrochloric acid and extracted with ether. The solvent is evaporated under vacuum, and the residue is triturated with benzene to give a 90% yield of colorless crystals, m.p. 131–133°.

(b) *Ethyl Acetamidocyanoacetate-2-C^{14}.* Ethyl cyanohydroxyiminoacetate-2-C^{14} (2.13 g.) in 50 ml. of acetic anhydride is hydrogenated at room temperature and atmospheric pressure over 1.0 g. of platinum-on-charcoal catalyst (5%) with vigorous shaking (Note 1). After the absorption of two molar amounts of gas (Note 2), the mixture is filtered through carbon with thorough washing with isopropyl alcohol. The solvent is evaporated at 40° under reduced pressure, and the residue is washed with ether. The yield is 2.17 g. (85%), m.p. 129–130° (Note 3).

B. Notes

1. The reductive acetylation of ethyl cyanohydroxyiminoacetate has been effected with: 1) zinc dust in acetic acid-acetic anhydride mixture, 60% yield;[2] 2) aluminum amalgam and acetic anhydride;[3] and 3) sodium hydrosulfite and acetic anhydride.[4] Hydrogenation[5] over palladium-charcoal catalyst (5%) gives yields of 45–75%, while platinum oxide catalyst gives 60–75%. Neither platinum nor palladium supported on asbestos gives any product, and Raney nickel is unsatisfactory.

2. With 5% palladium-charcoal, or Raney nickel catalysts, four moles of hydrogen are rapidly consumed with no diminution of rate after two moles, such as is found with 5% platinum-charcoal or platinum oxide catalysts.

3. Ethyl acetamidocyanoacetate has been found to be an advantageous intermediate in a general method for synthesizing α-amino acids[6] (alkylation followed by hydrolysis).

C. Other Preparations

Ethyl cyanohydroxyiminoacetate-2-C^{14} and ethyl acetamidocyanoacetate-2-C^{14} have been prepared by Bouthillier,[7] by essentially this same procedure with one variation—the hydroxyimino compound was reduced with zinc.

[1] H. R. Snyder and C. W. Smith, J. Am. Chem. Soc., *66*, 350 (1944).

[2] L. Light and Co., Ltd. and H. J. F. Weiss, Brit. 583,307; through Chem. Abstracts, *41*, 2747 (1947).

[3]V. Cerchez and C. Colesiu, Compt. rend., *194*, 1954 (1932).
[4]B. F. Tullar, U. S. 2,393,723; through Chem. Abstracts, *40*, 2465 (1946).
[5]M. Fields, D. E. Walz and S. Rothchild, J. Am. Chem. Soc., *73*, 1000 (1951).
[6]N. F. Albertson, J. Am. Chem. Soc., *68*, 450 (1946).
[7]L. P. Bouthillier and A. D'Iorio, Rev. can. biol., *9*, 382 (1950).

ETHYL ACETOACETATE-1-C^{13}

METHOD I

$$BrCH_2C^*OOC_2H_5 \xrightarrow[Mg]{CH_3COOCH_3} CH_3COCH_2C^*OOC_2H_5 \xrightarrow{NaOH} CH_3COCH_2C^*OONa$$
(a) (b)

W. Sakami, W. E. Evans and S. Gurin, J. Am. Chem. Soc., *69*, 1110 (1947).

A. Procedure[1]

(a) *Ethyl Acetoacetate-1-C^{13}* (see ethyl acetoacetate-3-C^{14}, Other Preparations). To a stirred mixture of 5.0 g. of methyl acetate, 15 ml. of absolute ether and 3 g. of magnesium turnings, under dry nitrogen, is added dropwise 10.5 g. of ethyl bromoacetate-1-C^{13}. The mixture is warmed to start the reaction, then maintained at a gentle boil by control of the addition rate. The product is obtained by hydrolysis of the Grignard reaction mixture, ether extraction, and distillation.

(b) *Sodium Acetoacetate-1-C^{13}*. The ester is hydrolyzed in the manner described for sodium acetoacetate-1,3-C_2^{13}. Degradation by the procedure of Lorber[2] forms carbon-C^{13} dioxide containing 0.86 atom per cent excess C^{13}, while the acetone contains only 0.02 atom per cent excess.

Ethyl acetoacetate-4-C^{14} is prepared[3] by condensing methyl acetate-2-C^{14} with ethyl bromoacetate by essentially the same procedure. Degradation studies (see ethyl acetoacetate-1-C^{14}, Note 4) show that 5–20% of the total activity is found at C-2.

METHOD II

$$Ag_2C^*O_3 \xrightarrow{C_2H_5I} (C_2H_5O)_2C^*O \xrightarrow[Na]{(CH_3)_2CO} CH_3COCH_2C^*OOC_2H_5$$
(a) (b)

M. Blecher and S. Gurin, J. Biol. Chem., *209*, 953 (1954).

A. Procedure

(a) *Ethyl Carbonate-C^{14}*. A mixture of 36.8 g. (133 mmoles) of dry silver carbonate-C^{14} (Note 1) and 22 ml. (267 mmoles) of redistilled

ethyl iodide (b.p. 72°) is stirred under a reflux condenser for 2 hours at room temperature with occasional cooling as necessary. The solution is fractionally distilled and the product is redistilled; yield 5.3 g. (34% based on silver carbonate), b.p. 128.5°.

(b) *Ethyl Acetoacetate-1-C^{14}*. Acetone (2.5 g., 43 mmoles) is carbethoxylated with 14.3 g. (129 mmoles) of ethyl carbonate-C^{14} by a suitable adaptation of the method of Lux,[4] which follows.

To a refluxing mixture of 12 g. of powdered sodium, 50 ml. of ether (Note 2) and 200 ml. of ethyl carbonate is added 40 ml. of acetone during 1–2 hours. The mixture is heated for 1 hour, cooled, and acidified with 50 ml. of glacial acetic acid diluted with 5 parts of water. The ester layer is separated, and the aqueous layer is saturated with sodium chloride and extracted with ether. The combined ester and extract is concentrated under reduced pressure and fractionally distilled; yield 25–28 g. (b.p. 60–70° at 11 mm.), 38–41% based on acetone.

The isotopic product is isolated from the acetone- and ether-free distillate by chilling, shaking with 25 ml. of ice-cold 1 *N* sodium hydroxide, quickly separating the aqueous solution of sodium enolate and adjusting to pH 8 with 1 *N* hydrochloric acid. The product is converted to the copper enol acetoacetate-1-C^{14} by the method described for ethyl acetoacetate-3-C^{14}, Method II; yield 1.25 g. (18% based on acetone), m.p. 195–196°. The yield of purified ethyl acetoacetate-1-C^{14} is 0.79 g. (79% based on the copper enolate), and the purity varies from 86 to 95% (Note 3). Degradation studies indicate that there is no isotopic rearrangement (Note 4).

B. Notes

1. The starting material is prepared from sodium carbonate-C^{14} by the method of Joulin.[5]

2. Substitution of benzene for the ether has no detectable influence, but heating at 100° without a low-boiling solvent reduces the yield.

3. Purity is determined by the manometric method of Edson[6] for catalytically decarboxylating β-keto acids in the presence of *o*-phenylenediamine citrate or aniline citrate.

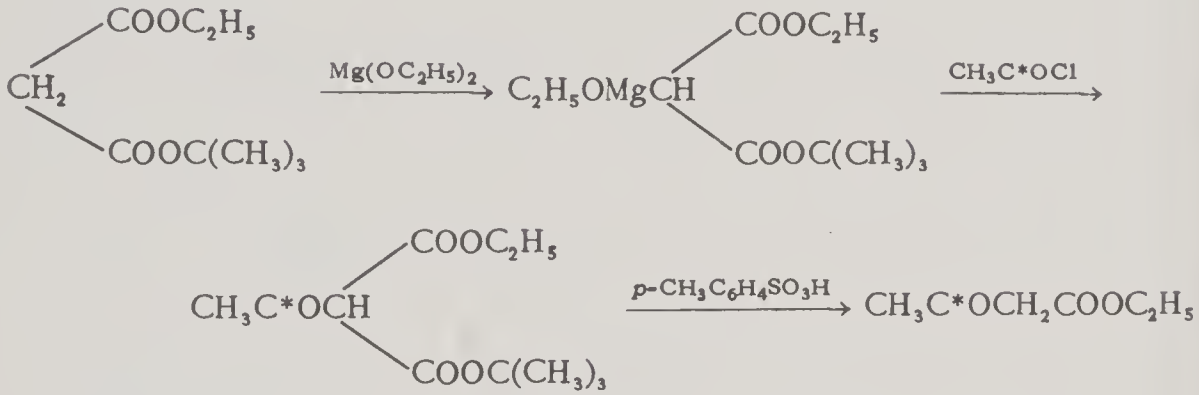
4. The acetoacetic-1-C^{14} acid is decarboxylated with Deniges reagent[6,7] (sulfuric acid-mercuric sulfate solution), forming an acetone-mercury complex (C-2,3,4). A degradation[4] by oxidation with acid-permanganate at low temperature yields acetic acid (C-3,4), formic acid (C-2) and carbonic acid (C-1).

[1]A. C. Rottinger and F. Wenzel, Monatsh., *34*, 1867 (1913).
[2]L. Lorber, Biochem. Z., *181*, 366 (1927).
[3]M. Blecher and S. Gurin, J. Biol. Chem., *209*, 953 (1954).
[4]H. Lux, Ber., *62*, 1824 (1929).
[5]L. Joulin, Ann. chim. et phys., (4) *30*, 248 (1873).

[6]N. L. Edson, Biochem. J., *29*, 2082 (1935).
[7]S. Weinhouse and R. H. Millington, J. Biol. Chem., *181*, 645 (1949).

ETHYL ACETOACETATE-3-C^{14}

METHOD I

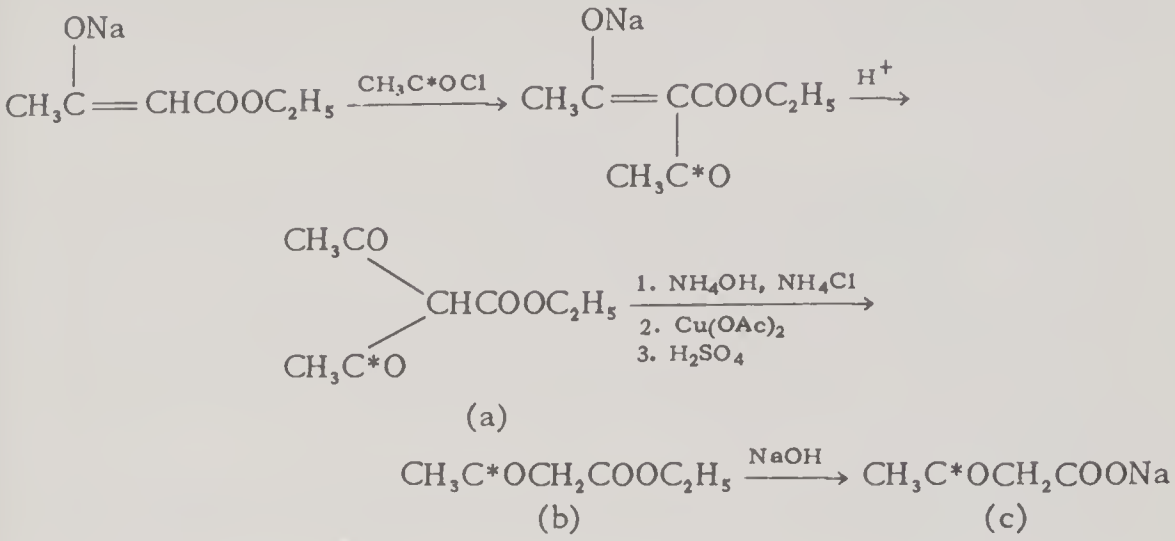


W. G. Dauben and H. L. Bradlow, J. Am. Chem. Soc., *74*, 5204 (1952).

A. Procedure (Note 1)

A mixture of 2.87 g. of magnesium, 100 ml. of absolute ethanol, 20 ml. of xylene and 2 ml. of carbon tetrachloride is refluxed for 12 hours. The mixture is evaporated to dryness at reduced pressure on a steam-bath, the removal of all alcohol being ensured by the evaporation of several portions of added benzene. The residue is heated for 3 hours at 100° under reduced pressure, then cooled and stirred with 40 ml. of absolute ether. To the vigorously stirred mixture is added dropwise 22.4 g. of ethyl *t*-butyl malonate,[1] and the mixture is refluxed until solution is complete. An anhydrous ethereal solution (25 ml.) of acetyl-1-C^{14} chloride, prepared from 10.8 g. of sodium acetate-1-C^{14}, is added dropwise with stirring. The mixture is refluxed for 30 minutes, cooled, diluted with water and acidified with dilute sulfuric acid. The aqueous solution is separated and extracted with ether. The combined extract is evaporated, and the residue is dissolved in 100 ml. of benzene and dried azeotropically by distillation of a portion of the solvent. The solution is treated with 0.75 g. of *p*-toluenesulfonic acid and refluxed for 1.5 hours (Note 2), then cooled, and washed with saturated sodium bicarbonate and saturated sodium chloride solutions. The product is collected by fractional distillation through an 18-inch column; yield 12.2 g. (71.3% based on sodium acetate-1-C^{14}), b.p. 180° (Note 3).

METHOD II



G. L. Curran, J. Biol. Chem., *191*, 775 (1951).

A. Procedure

(a) *Ethyl 2-Acetyl-1-C*^{14}*-acetoacetate* (Note 4). Acetyl-1-C^{14} chloride (2.0 g., 26 mmoles) is condensed with 4.0 g. (30 mmoles) of the sodium enolate of ethyl acetoacetate (Note 5); see threonine-4-C^{14}, or ethyl diaceto-2-C$_1^{14}$-acetate. Acidification of the precipitated 2-acetyl-1-C^{14}-sodiumacetoacetate gives the product (Note 6).

(b) *Ethyl Acetoacetate-3-C*^{14}. The substituted acetoacetic ester is hydrolyzed in ammonium chloride-ammonium hydroxide mixture, according to the procedure of Shriner.[2] The product is extracted from the hydrolysis mixture with ether, and the extract is washed with water (until neutral to litmus) and evaporated in a stream of dry air. The residue is treated with 100 ml. of a filtered, saturated solution of copper acetate, and the mixture is adjusted to pH 6.5 with dilute ammonium hydroxide and stored for 30 minutes. The precipitate is collected, washed with water and dried over calcium chloride. The yield of ethyl acetoacetate-3-C^{14} copper enolate is 2.58 g., m.p. 195°, 62% based on acetyl chloride (Notes 6 and 7). A mixture of 0.5 g. (1.6 mmoles) of the copper derivative, 10 ml. of 25% sulfuric acid and 20 ml. of ether is shaken until solution takes place; then the aqueous layer is separated and extracted with ether. The combined ether solution is washed with three portions of saturated sodium sulfate solution, dried over sodium sulfate and concentrated, and the product is distilled; yield 0.255 g., b.p. 180° (760 mm.).

(c) *Sodium Acetoacetate-3-C*^{14}. The ester is mixed with the calculated amount of *N* sodium hydroxide and stored at 0–4° for 24 hours. The

product is isolated by lyophilization. Degradation studies (Note 8) show that 2–3% of the activity of the pure ester is present as sodium acetate, after saponification.

B. Notes

1. The procedure is adapted from the method of Breslow, Baumgarten and Hauser[1] for synthesizing β-keto esters.

2. The elimination of isobutylene and decarboxylation of the acylated malonic half-ester is effected by catalytic amounts of acid.

3. The ester is readily hydrolyzed to form sodium acetoacetate-3-C^{13}, see Method II.

4. The procedure is according to Michael and Carlson.[3]

5. Sodium acetoacetic ester may be prepared by the procedure of Shriner, Schmidt and Roll.[2]

6. The separation of ethyl acetoacetate (b.p. 96° at 40 mm.) and ethyl 2-acetylacetoacetate (b.p. 120° at 39 mm.) is effected through the copper enolates. The distilled mixture is treated with saturated copper acetate solution, and the precipitated ethyl 2-acetylacetoacetate copper enolate is collected; yield 85% from pure 2-acetylacetoacetic ester. The ethyl acetoacetate copper enolate is precipitated from the filtrate by neutralizing with dilute ammonia; yield 95% from pure acetoacetic ester.

7. The molar specific activity of the acetoacetic ester is one-half of that of the starting acetyl chloride, but a considerable amount of acetic-1-C^{14} acid can be recovered from the reaction mixture, see threonine-4-C^{14}.

8. Sodium acetoacetate-3-C^{14} is decarboxylated with 25% sulfuric acid or with aniline-citrate reagent,[4] carrier sodium acetate is added, and the acetic acid is collected by distillation and converted to the silver salt for assay.

C. Other Preparations

Ethyl acetoacetate-3-C^{14} has been prepared by Sakami,[5] in 19% yield (88% purity) based on potassium acetate, by a modification of the reaction of Röttinger and Wenzel.[6] The product contains isotope at C-3 only when the Grignard reagent from ethyl bromoacetate is prepared in the presence of methyl acetate-1-C^{14}. Only nonisotopic ethyl acetoacetate is formed when the Grignard reagent is formed and then condensed with isotopic methyl acetate by the original procedure. Dauben has reported the preparation as being erratic; also see ethyl acetoacetate-1-C^{13}.

The preparation of ethyl 2-acetyl-1-C^{14}-acetoacetate and ethyl acetoacetate-3-C^{14} (b.p. 69–70° at 21 mm.) in 87% and 22% radiochemical

yield, respectively, based on acetyl-1-C^{14} chloride has been described by Davis,[7] employing a modification of Method II.

The preparation of ethyl acetoacetate-1,3-C_2^{14} by condensation of ethyl acetate-1-C^{14} under the influence of triphenylmethylsodium has been reported by Langdon.[8]

[1]D. S. Breslow, E. Baumgarten and C. R. Hauser, J. Am. Chem. Soc., *66*, 1286 (1944); B. Riegel and W. M. Lilienfeld, *ibid.*, *67*, 1273 (1945).

[2]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 266.

[3]A. Michael and G. H. Carlson, J. Am. Chem. Soc., *57*, 165 (1935).

[4]N. L. Edson, Biochem. J., *29*, 2082 (1935).

[5]W. Sakami, W. E. Evans and S. Gurin, J. Am. Chem. Soc., *69*, 1110 (1947).

[6]A. C. Röttinger and F. Wenzel, Monatsh., *34*, 1867 (1913).

[7]H. W. Davis, E. Grovenstein, Jr., and O. K. Neville, J. Am. Chem. Soc., *75*, 3304 (1953).

[8]R. G. Langdon and K. Bloch, J. Biol. Chem., *200*, 135 (1953); *Organic Reactions*, Vol. I, Wiley, New York, 1942, p. 266.

ETHYL OXALACETATE-3-C^{14}

METHOD I

$$C^*H_3COOC_2H_5 \xrightarrow[NaOC_2H_5]{(COOC_2H_5)_2} \left[\begin{matrix} C^*HCOOC_2H_5 \\ | \\ COCOOC_2H_5 \end{matrix}\right]^- Na^+ \xrightarrow{H_2SO_4} \begin{matrix} C^*H_2COOC_2H_5 \\ | \\ COCOOC_2H_5 \end{matrix}$$

G. A. Ropp, J. Am. Chem. Soc., *72*, 4459 (1950).

A. Procedure (Note 1)

Ethyl acetate-2-C^{14} (0.286 g., 3.25 mmoles) is condensed at 70° with 3.24 mmoles of ethyl oxalate in the presence of 3.1 mmoles of sodium dissolved in 2 ml. of absolute ethanol. The reaction mixture is distilled to dryness at 0.1 μ (Note 2), and the residual sodium salt (Note 3) is decomposed by stirring at 0° with 7 ml. of benzene and 1.3 ml. of 2.85 *N* sulfuric acid. The benzene layer is separated, washed with three 2.5-ml. protions of water (Note 4) and vacuum-distilled (Note 5); yield of crude product is 0.309 g., 51% based on ethyl acetate.

METHOD II

$$C^*H_3COOC_2H_5 \xrightarrow[NaOCH_3]{(COOC_2H_5)_2} \begin{matrix} C^*H_2COOC_2H_5 \\ | \\ COCOOC_2H_5 \end{matrix}$$

R. M. Lemmon, Atomic Energy Commission Report, AECU-452; Nuc. Sci. Abstracts, 3, 1626 (1949).

A. Procedure (Note 6)

To a stirred solution of 40 mmoles of sodium methoxide and 30 mmoles of ethyl oxalate in 30 ml. of absolute ether is added 20 mmoles of ethyl acetate-2-C^{14} in a few ml. of ether. The solution is refluxed for 16 hours, then poured onto 20 g. of ice-sulfuric acid mixture, and the acid solution is extracted continuously with ether overnight. The ether solution is concentrated to 25 ml. and extracted with small portions of 10% potassium carbonate solution until the ether phase no longer gives a positive test for enol (Note 7). The combined alkaline solution is acidified with sulfuric acid, and the crude product is isolated by continuous ether extraction for 10 hours, followed by evaporation of the solvent.

B. Notes

1. The procedure is a modification of that described by Jorgensen[1] and is based on the method of Adickes and Andresen[2] for preparing α-keto acids; see potassium pyruvate-3-C^{14}; also see Hauser and Hudson.[3]

2. The distillate contains 26.7% of the starting activity.

3. The yield of sodium salt has been reported[3] as 60–80%.

4. The combined aqueous solutions contain 3.4% of the starting activity.

5. The distilled benzene contains 4.8% of the starting activity.

6. The procedure is an adaptation of the method of Adickes and Andresen.[2]

7. A 1-ml. aliquot of the ether solution is diluted with 2 ml. of alcohol and treated with 4–5 drops of 1% ferric chloride solution, producing a purple color in the presence of enol.

C. Other Preparations

In a similar manner have been prepared: ethyl oxalacetate-3-C^{14} by Jorgensen[1] (Method II); and ethyl oxalacetate-4-C^{14} by Benson,[4] and Wang[5] (yield 87% of the potassium salt, based on sodium acetate-1-C^{14}) by Method II.

Ethyl oxalacetate-3-C^{14} has been prepared[6] by adding ethyl acetate-2-C^{14} dropwise to a mixture of ethyl oxalate, ether and sodium wire.[7] After 12 hours the precipitated sodium salt is collected and converted to the free ester by shaking with 5 *N* hydrochloric acid and ether, drying of the extract over magnesium sulfate and evaporating. The ethyl oxalacetate-3-C^{14} was converted[6] to ethyl 2-chlorofumarate-3-C^{14} by the method of Michael,[8] and then to 2-aminofumaramide-3-C^{14} by stirring the oily chloro ester with concentrated ammonium hydroxide [if dilute ammonia (3.7 *N*) is used, the deposited product is ethyl chlorofumarate-3-C^{14}, m.p. 100°] until a

crystalline product separated, m.p. 206–208° (dec.) from methanol.[9] The molecular extinctions and ultraviolet absorption of 2-aminofumaramide in neutral and alkaline solutions are described by Cooper. Below pH 5 the compound undergoes rearrangement and the ultraviolet absorption nearly disappears. The compound is converted to aspartic-3-C^{14} acid by hydrogenation and hydrolysis.

[1]E. C. Jorgensen, J. A. Bassham, M. Calvin and B. M. Tolbert, J. Am. Chem. Soc., *74*, 2418 (1952).
[2]F. Adickes and G. Andresen, Ann., *555*, 41 (1943).
[3]*Organic Reactions*, Vol. I, Wiley, New York, 1942, p. 292.
[4]A. A. Benson, J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas and W. Stepka, J. Am. Chem. Soc., *72*, 1710 (1950).
[5]S. C. Wang, T. Winnick and J. P. Hummel, J. Am. Chem. Soc., *73*, 2390 (1951).
[6]C. Cooper, R. Wu and D. W. Wilson, J. Biol. Chem., *216*, 37 (1955).
[7]W. Wislicenus, Ber., *19*, 3225 (1886).
[8]A. Michael and C. Schulthess, J. prakt. Chem., *46*, 236 (1892).
[9]W. H. Perkin, J. Chem. Soc., *53*, 695 (1888); R. T. Mamert, Bull. soc. chim. France, (3) *11*, 96 (1894).

ETHYL 5-CYANO-C^{14}-2-HYDROXIMINOVALERATE

$$Cl(CH_2)_3Br \xrightarrow{KC^*N} Cl(CH_2)_3C^*N \xrightarrow[NaOC_2H_5]{CH_2(COOC_2H_5)_2}$$

(a)

$$NC^*(CH_2)_3CH(COOC_2H_5)_2 \xrightarrow[NaOC_2H_5]{C_2H_5ONO} NC^*(CH_2)_3C(=NOH)COOC_2H_5$$

(b) (c)

H. Borsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighley and P. H. Lowy, J. Biol. Chem., *176*, 1383 (1948).

A. Procedure (Note 1)

(a) *4-Chlorobutyronitrile-1-C^{14}* (Note 2). A solution of 5.95 g. of potassium cyanide-C^{14} in 4 ml. of water and 28 ml. of absolute ethanol is titrated at 0° to pH 10.8 with 6 *N* hydrochloric acid (3.8 ml.); then the glass electrodes are rinsed with 2 ml. of water. The stirred solution is refluxed for 5.5 hours with 19.2 g. of 1-bromo-3-chloropropane,[1] cooled and diluted with 45 ml. of water, and the two layers are separated. A chloroform extract (12 ml.) of the upper layer is added to the lower layer, and the mixture is washed with 10 ml. of 16% calcium chloride solution

and 3 ml. of water, and dried over fused calcium chloride. The solvent is distilled at atmospheric pressure, and the residue is fractionally vacuum-distilled; yield 1.535 g. (75%), b.p. 89–93° (24 mm.) (Note 3).

(b) *Ethyl (3-Cyano-C^{14}-propyl)malonate.* To a cooled solution of 1.0 g. of sodium in 10 ml. of absolute ethanol, contained in a Carius tube, are added 13.3 g. of ethyl malonate, and a mixture of 13.3 g. of ethyl malonate and 2.74 g. of 4-chlorobutyronitrile-1-C^{14}, followed by 13.3 g. of ethyl malonate. The mixture is heated in the sealed tube for 15 hours at 95–98°. Ethanol, ethyl malonate and unreacted nitrile are removed by steam distillation, and the residue is extracted with ether. The extract is dried for 15 minutes over potassium carbonate, filtered, concentrated and vacuum-distilled; yield 5.20 g. (86%), b.p. 128–131° (0.5 mm.) (Note 4).

(c) *Ethyl 5-Cyano-C^{14}-2-hydroximinovalerate.* An ice-cold mixture of 1.13 ml. of ethyl nitrite and 1.628 g. of ethyl (3-cyano-C^{14}-propyl)malonate is added dropwise to a solution of 0.166 g. of sodium in 2.64 ml. of absolute ethanol at −18°, and washed down with 0.66 ml. of cold ethanol. The mixture is stored for 21 hours at −15° to −10° (Dewar flask); then the solvent is evaporated under vacuum at room temperature. The residue is dissolved in 10 ml. of water and extracted with 5 ml. of ether, which is then washed with two 1-ml. portions of water. The combined aqueous solution is cooled, acidified with 10% sulfuric acid and extracted with ether. The extract is evaporated under vacuum and dried over sulfuric acid; yield of crystalline product 1.195 g., 90.5% (Note 5).

B. Notes

1. The procedure is nearly the same as that described by Olynyk[2] for preparing lysine-6-C^{14} by the method of Fischer.[3]

2. The procedure is a modification of the method of Allen.[4] Either the sodium or potassium salt is suitable.

3. Olynyk[2] has reported an isotopic yield of 75%, with yields of 82% in trial runs. The temperature range of the collected cut is wide so as to include any bromonitrile formed, which would be usable in the next step. The pure compound boils at 93–96° (26 mm.), and 194–197° (745 mm.).

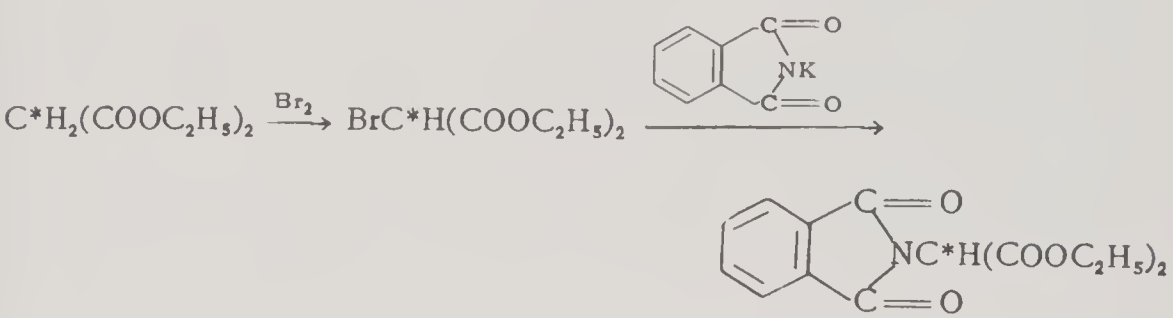
Glutaronitrile-1,5-C_2^{14}, isolated by fractional distillation as a by-product, has been hydrolyzed to glutaric-1,5-C_2^{14} acid by refluxing for 4 hours with concentrated hydrochloric acid. The aqueous solution was evaporated to dryness, and the crystalline product was isolated by benzene extraction (24 hours) in a Soxhlet apparatus. Further purification was achieved through preparation of piperazine monoglutarate-1,5-C_2^{14} in acetone, followed by decomposition of the salt with hydrochloric acid and extraction with ether. The glutaric-1,5-C_2^{14} acid was obtained in 94% recovery, m.p. 97–98°, neutralization equivalent 65.5 (calc. 66.0).

4. Olynyk[2] has reported a yield of 74%, b.p. 138–143° (1.1 mm.), obtained by conducting the isotopic reaction under reflux with alcohol-free sodium ethoxide, ether and excess ester. An attempt was made to prepare the compound by inverting steps (a) and (b), but the nitrilation of (3-chloropropyl)malonic ester proceeds slowly and with side reactions.

5. Olynyk[2] has obtained yields of 70–83% by a slight modification of this method.

[1]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, pp. 533 and 25.
[2]P. Olynyk, D. B. Camp, A. M. Griffith, S. Woislowski and R. W. Helmkamp, J. Org. Chem., *13*, 465 (1948).
[3]E. Fischer and F. Weigert, Ber., 35, 3772 (1902).
[4]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 156.
[5]M. Rothstein and L. L. Miller, J. Biol. Chem., *199*, 199 (1952).

ETHYL PHTHALIMIDOMALONATE-2-C^{14}



H. R. V. Arnstein, G. D. Hunter, H. M. Muir and A. Neuberger, J. Chem. Soc., *1952*, 1329.

A. Procedure (Note 1)

To a solution of 0.206 g. of ethyl malonate-2-C^{14} in 20 ml. of dry, freshly distilled carbon tetrachloride is added a small excess of bromine (0.212 g.) in 3.6 ml. of carbon tetrachloride. When the reaction appears to be complete (1 hour), 1.31 g. of carrier ethyl bromomalonate is added, and the solution is stored overnight. The solvent is evaporated at room temperature in a stream of dry air (Note 2). The residue is dissolved in 20 ml. of dry toluene and heated with a 10% excess of potassium phthalimide[1] (1.146 g.) in a sealed tube for 2 hours at 120–130°, and then at 140° for 2 hours. The mixture is filtered, and the residue is discarded after being extracted with benzene. The filtrates are evaporated to dryness, and the residue is combined with 1.06 g. of carrier ethyl phthalimidomalonate and recrystallized from ether-petroleum ether; yield 2.35 g. (Note 3).

B. Notes

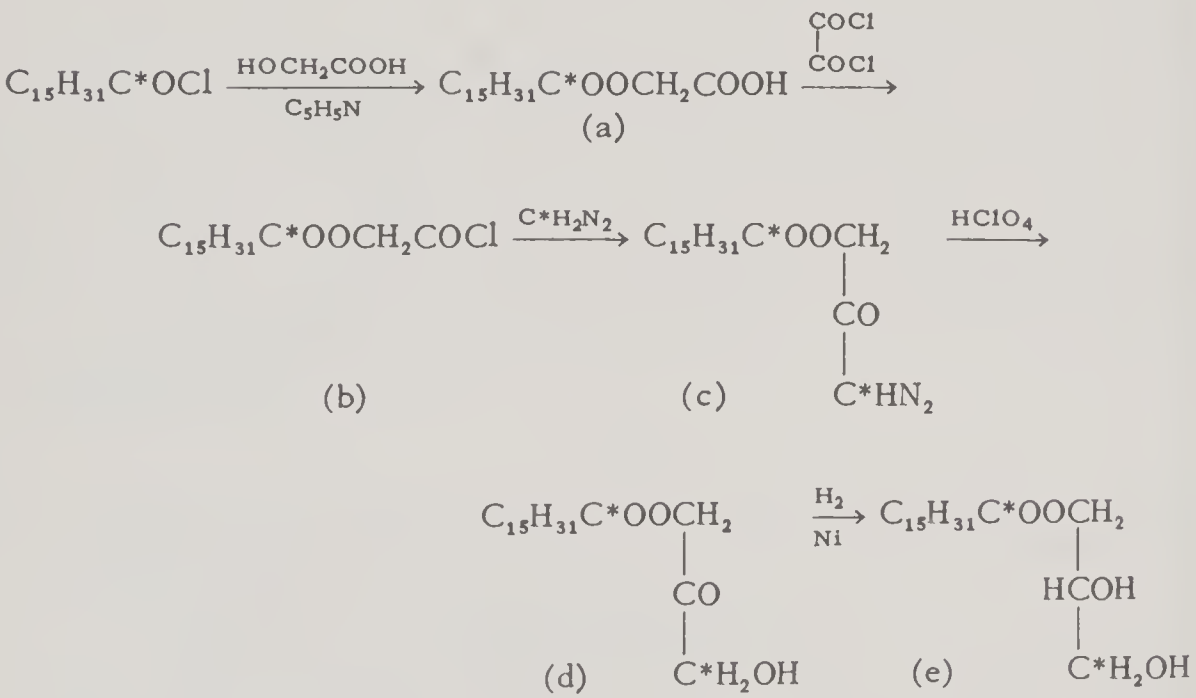
1. The procedure is adapted from the methods of Palmer and McWherter[2] and Osterberg.[3]

2. The yield of ethyl bromomalonate is 73–75% on the usual laboratory scale.

3. The yield of product from ethyl bromomalonate is 67–71% on the usual laboratory scale.

[1]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 119.
[2]*ibid.*, p. 245.
[3]*ibid.*, p. 271.

2,3-DIHYDROXYPROPYL-3-C^{14} HEXADECANOATE-1-C^{14}
(2,3-Dihydroxypropyl-3-C^{14} Palmitate-C^{14})



H. Schlenk, B. G. Lamp and B. W. DeHaas, J. Am. Chem. Soc., *74*, 2550 (1952).

A. Procedure (Note 1)

(a) *Hexadecanoyloxy-1-C^{14}-acetic Acid.* Palmitic-C^{14} acid is esterified by treating the acid chloride with glycolic acid in pyridine. The product melts at 83–84° after recrystallization from Skelly B (Note 2).

(b) *Hexadecanoyloxy-1-C^{14}-acetyl Chloride* (Note 3). The acid chloride is prepared by refluxing the acid with a 2-molar excess of oxalyl chloride and an equal weight of benzene. The mixture is evaporated to dryness, and the solid is used without further purification.

(c) *3-Diazo-1-hexadecanoyloxy-1-C^{14}-2-propanone-3-C^{14}*. To a stirred solution of 27.0 g. (81.4 mmoles) of the acid chloride in 400 ml. of ether, maintained at −2°, is added an ethereal solution of diazomethane-C^{14} during 1 hour (Note 4). The excess of diazomethane is removed, and the solution is concentrated to one-third volume by flushing with air under slightly diminished pressure. The product is crystallized at 4°, collected and recrystallized from ether; yield 16.6 g., m.p. 57–59° (Note 5).

(d) *1-Hexadecanoyloxy-1-C^{14}-3-hydroxy-2-propanone-3-C^{14}* (Note 6). A solution of 4.0 g. of the diazoketone in 40 ml. of dioxane, and a solution of 3 g. of perchloric acid in 20 ml. of water and 40 ml. of dioxane are poured together at 80°. The mixture is heated on a water-bath to complete the evolution of nitrogen (1 minute), then cooled rapidly and poured into 350 ml. of ice water. The collected, wet precipitate is dissolved in ether, and the solution is washed to remove acid, dried over sodium sulfate and concentrated to 30 ml. The warm solution is poured into 100 ml. of Skelly F, and the mixture is stored overnight in a refrigerator. The crude product is collected; yield 2.4 g., m.p. between 58° and 93° (Note 7).

(e) *2,3-Dihydroxypropyl-3-C^{14} Hexadecanoate-1-C^{14}, (2,3-Dihydroxypropyl-3-C^{14} Palmitate-C^{14}), (C_2^{14}-α-Monopalmitin)*. The crude (Note 8) 1-hexadecanoyloxy-1-C^{14}-3-hydroxy-2-propanone-3-C^{14} (0.82 g.) in 50 ml. of tetrahydrofuran is hydrogenated at 25 p.s.i. over freshly prepared Raney nickel (Note 9). When the reaction is complete (2 hours), the catalyst is removed by centrifugation, and the solution is evaporated to dryness. The product is recrystallized from cyclohexane-ether, m.p. 72–74°.

B. Notes

1. The isotopic preparations are mentioned by Reiser and Williams,[1] but the directions are those of the nonisotopic preparations. Since the method permits synthesizing glycerides by successively forming the carbinol groups of labeled glycerol, the method makes available glycerides in which the positions of the individual fatty acids (up to three) with respect to the label are known. It has been demonstrated by Doerschuk[2] that the acyl group transfer in the rearrangement[3] of partially acylated glycerides is intramolecular.

2. The yield is satisfactory, and the method is preferable to that of reacting chloracetic acid with potassium palmitate.

3. The method is that of Adams and Ulich.[4]

4. A precipitate forms which is soluble at room temperature.

5. The yield is approximately 60%, and some 3-chloro-1-hexadecanoyloxy-[1-C^{14}]-2-propanone-[3-C^{14}] is formed, despite the fact that the Arndt-Eistert reaction[5] is carried out with a large excess of diazome-

thane. Usually, it is advantageous to add the acid chloride to the excess of diazomethane. The diazoketone is stable for weeks in the dark at refrigerator temperature.

6. The usual hydrolysis of diazoketones to hydroxymethyl ketones in the presence of dilute sulfuric acid leads to the formation of a by-product, 3-palmitoxyacetonyl sulfate.

7. 1-Hexadecanoyloxy-3-hydroxy-2-propanone (m.p. 63°) forms a dimer (m.p. 93°) upon standing, but recrystallization from boiling ethanol converts the latter to the monomer. Hot Skelly B extracts the monomer from a mixture of the two.

8. Both the monomer and the dimer are used.

9. The alloy[6] (10 g.) is placed in a hydrogenation flask, similar to the Parr apparatus, and treated[7,8] with sodium hydroxide and water until the washings are approximately pH 9; then the water is replaced by tetrahydrofuran.

[1]R. Reiser and M. C. Williams, J. Biol. Chem., *202*, 815 (1953).

[2]A. P. Doerschuk, J. Am. Chem. Soc., *74*, 4202 (1952).

[3]H. Hibbert and M. E. Greig, Can. J. Research, *4*, 254 (1931); B. F. Stimmel and C. G. King, J. Am. Chem. Soc., *56*, 1724 (1934).

[4]R. Adams and L. H. Ulich, J. Am. Chem. Soc., *42*, 599 (1920).

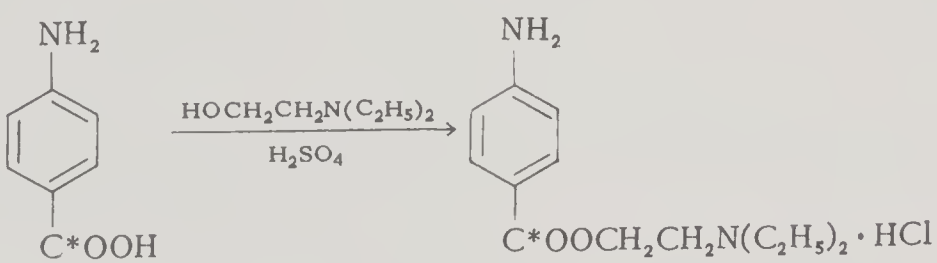
[5]*Newer Methods of Preparative Organic Chemistry*, Interscience Publishers, New York, 1948, p. 537; *Organic Reactions*, Vol. I, Wiley, New York, 1942, Chapt. 2.

[6]Central Scientific Co., Chicago, Illinois.

[7]*Organic Syntheses*, Vol. 21, Wiley, New York, 1941, p. 15.

[8]Reference 3, p. 61.

2-DIETHYLAMINOETHYL *p*-AMINOBENZOATE-C^{14} HYDROCHLORIDE (C_1^{14}-Procaine Hydrochloride)



H. G. Mandel and C. L. Brown, Department of Pharmacology, The George Washington University School of Medicine, Washington, D. C., private communication.

A. Procedure (Note 1)

To an ice-cooled solution of 1 g. (7.3 mmoles) of *p*-aminobenzoic-C^{14} acid in 7 ml. of concentrated sulfuric acid is added slowly 2.3 ml. (19

mmoles) of 2-diethylaminoethanol. The mixture is refluxed at 85° for 7 hours, then cooled to 0°, neutralized with concentrated ammonia, and extracted with several portions of ether (Note 2). The combined extracts are dried over sodium sulfate (overnight), and the product is precipitated by the dropwise addition of a solution of dry hydrogen chloride in ether. The yield of colorless crystalline product is 0.9 g. (45%). The melting point, after two recrystallizations from absolute ethanol and ether, is 155–156°.

B. Notes

1. The procedure is an adaptation of the method of Einhorn and Uhlfelder.[1]

2. The unreacted *p*-aminobenzoic-C^{14} acid is recovered almost quantitatively by neutralization and evaporation of the aqueous solution remaining after extraction of the ester.

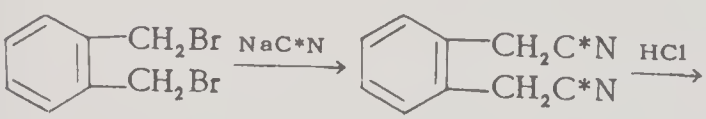
C. Other Preparations

The preparation of ethyl C^{14}-*p*-aminobenzoate, C^{14}-procaine and C^{14}-cocaine has been reported by Markova.[2]

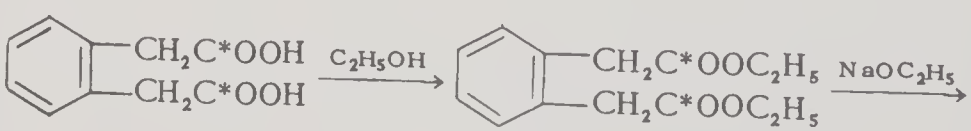
[1] A. Einhorn and E. Uhlfelder, Ann., *371*, 131 (1909); Chem. Zentr., *81*, 1015 (1910).

[2] Y. V. Markova, L. N. Zenkova and M. N. Shchukina, Zhur. Obshcheĭ Khim., *25*, 1383 (1955); Nuc. Sci. Abstracts, *9*, 7315 (1955).

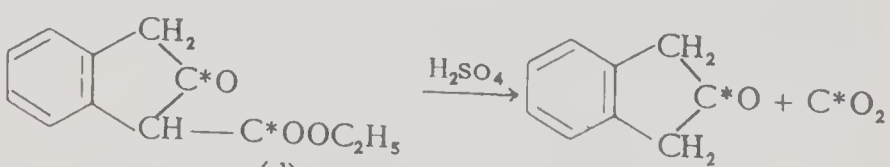
ETHYL 2-OXO-1-INDANCARBOXYLATE-C^{14}-2-C^{14}



(a)



(b) (c)



(d)

W. L. Carrick and A. Fry, J. Am. Chem. Soc., 77, 4381 (1955).

A. Procedure

(a) *o-Benzenediacetonitrile-C_2^{14}.* α,α′-Dibromo-o-xylene[1] is reacted with sodium cyanide-C^{14} by suitable adaptation of the method of Moore and Thorpe,[2] which follows.

A concentrated aqueous solution of potassium cyanide (10% excess) is diluted with 3 volumes of alcohol and treated at the boiling point with finely ground α,α′-dibromo-o-xylene in portions. The mixture is stored for 30 minutes, then diluted with water, and the oil is extracted into ether solution which is dried, concentrated and stored to crystallize the product. The mother liquor yields a second crop of crystals upon dilution with an equal volume of alcohol. o-Benzenediacetonitrile is recrystallized from methanol, m.p. 60° (Note 1).

(b) *o-Benzenediacetic-C_2^{14} Acid, (o-Phenylenediacetic-1-C_2^{14} Acid).* The nitrile is hydrolyzed by the procedure of v. Braun,[3] which follows.

The isotopic product is purified by partition between ether and sodium hydroxide solution, treatment with carbon, and repeated recrystallization alternately from water and from ether-petroleum ether; m.p. 150–151°.

A mixture of the nitrile and a five-fold excess of concentrated hydrochloric acid is heated in a sealed tube for 5 hours at 110° and evaporated to dryness. The yield is quantitative and the product is analytically pure.

(c) *Ethyl o-Benzenediacetate-C_2^{14}.* The acid is refluxed for 3 hours in absolute ethanol solution saturated with dry hydrogen chloride. The solution is concentrated to about one-third volume under reduced pressure, then diluted with cold water and extracted with ether. The extract is washed with water, 10% sodium bicarbonate solution and water, then dried over potassium carbonate, concentrated and fractionally distilled; b.p. 193–194° (15 mm.).

(d) *Ethyl 2-Oxo-1-indancarboxylate-C^{14}-2-C^{14}, (Ethyl 2-Hydrindone-2-C^{14}-1-carboxylate-C^{14})* (Note 2). To a solution of 0.42 g. (18 mmoles) of sodium in 15 ml. of absolute ethanol is added 4.5 g. (18 mmoles) of ethyl o-benzenediacetate-C_2^{14} in 15 ml. of absolute alcohol. The solution is refluxed for 15 minutes, then cooled, diluted with 60–70 ml. of cold water and acidified with dilute hydrochloric acid. The product is collected on a filter, washed with water and recrystallized from alcohol; yield 2.58 g. (70%), m.p. 65°. The molar specific activity agrees with that of the starting material. Degradation studies (Note 3) indicate that 51.3% of the activity is located in the carboxyl group.

B. Notes

1. The labile needles separating below 18° pass into a stable, prismatic form with some change in volume and a loud report.

2. This preparation is incidental to a tracer study of the Dieckmann condensation—the intramolecular condensation of an ester of a dibasic acid to produce a cyclic β-ketoester.[4] The rate-determining step in a reaction is located by determining whether or not there is an isotope effect in the reaction with molecules successively labeled in different positions. The observance of large isotope effects with the same compound in both of two experiments, which differ only in the position of labeling, indicates that the rate-controlling step must involve both of these positions. An intramolecular isotope effect of 5.6% is observed with ethyl *o*-benzenediacetate-C_2^{14}, and an intermolecular isotope effect of about 8.5% is observed with both ethyl *o*-benzenediacetate-α-C_2^{14} and ethyl *o*-benzenediacetate-C_2^{14}, when the condensation is carried out in alcoholic sodium ethoxide. Since the only step involving both labeled positions, in this case, is C–C bond formation, it is concluded that this must be the rate-determining step of the reaction.

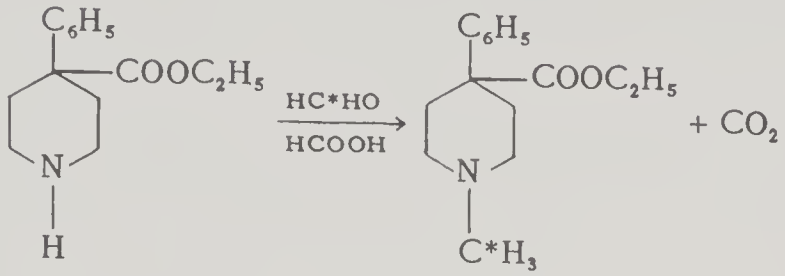
3. Directions are given by Carrick and Fry for decarboxylating[5] the ester by heating with 20% sulfuric acid for 5 hours on a steam-bath in a nitrogen sweep system. The carbon-C^{14} dioxide is collected as barium carbonate-C^{14}, 100.1%. The reaction mixture is diluted with alcohol, then heated to solution and cooled to 50° to crystallize a small amount of 1-(2-indanylidene-2-C^{14})-2-indanone-2-C^{14} (<10%), m.p. 170–175°.[5] The filtrate is cooled to 10° to crystallize the 2-indanone-2-C^{14}, m.p. 57°. The molar specific activities of the carbon-C^{14} dioxide and 2-indanone-2-C^{14} are 51.3% and 48.7%, respectively, of that of the starting material.

C. Other Preparations

In a similar manner from α,α′-dibromo-*o*-xylene-α-C^{14} are prepared: *o*-benzenediacetonitrile-α-C^{14}, *o*-benzenediacetic-α-C^{14} acid, ethyl *o*-benzenediacetate-α-C^{14}, ethyl 2-oxo-1-indancarboxylate-1,3-$C^{14}_{1/2}$ and 2-indanone-1-C^{14}. The starting material was prepared from *o*-toluic-C^{14} acid: directions are given for reducing the acid with lithium aluminum hydride, forming *o*-methylbenzyl-α-C^{14} alcohol; yield 80%, b.p. 210–217° (730 mm.). Treatment with phosphorus tribromide gave *o*-methylbenzyl-α-C^{14} bromide (69% yield). Halogenation with the stoichiometric amount of bromine[1] gave α,α′-dibromo-*o*-xylene-α-C^{14}, which was recrystallized from chloroform; yield 70%, m.p. 90–92°.

[1]W. H. Perkin, Jr. and A. F. Titley, J. Chem. Soc., *121*, 1562 (1922).
[2]C. W. Moore and J. F. Thorpe, J. Chem. Soc., *93*, 165 (1908).
[3]J. v. Braun, O. Kruber and E. Danziger, Ber., *49*, 2642 (1916).
[4]*Organic Reactions*, Vol. I, Wiley, New York, 1942, p. 274.
[5]F. Heusler and H. Schieffer, Ber., *32*, 32 (1899).

ETHYL 1-METHYL-C_1^{14}-4-PHENYLISONIPECOTATE (C_1^{14}-Demarol)



W. Tarpey, H. Hauptmann, B. M. Tolbert and H. Rapoport, J. Am. Chem. Soc., 72, 5126 (1950).

A. Procedure (Note 1)

The apparatus consists of a 10-ml., three-necked, pear-shaped flask equipped with a micro dropper, a capillary tube inlet for nitrogen, a reflux condenser connected to a spiral, Dry Ice-cooled trap, and a spiral bubbler filled with 15 ml. of 2 *N* carbonate-free sodium hydroxide and protected by a guard tube filled with soda lime; see Figure III, 3.

To 1.070 g. (4.6 mmoles) of ethyl 4-phenylisonepecotate at 0° is added 0.547 g. of 90% formic acid (10.7 mmoles), and the system is swept with nitrogen. Aqueous formaldehyde-C^{14} (0.598 g. of 21% solution, 4.2 mmoles) is added, and the dropper is rinsed with 0.4 ml. of water. As the mixture is warmed slowly to 100°, a rapid evolution of carbon dioxide starts at 85° and lasts for 20 minutes. The mixture is re-

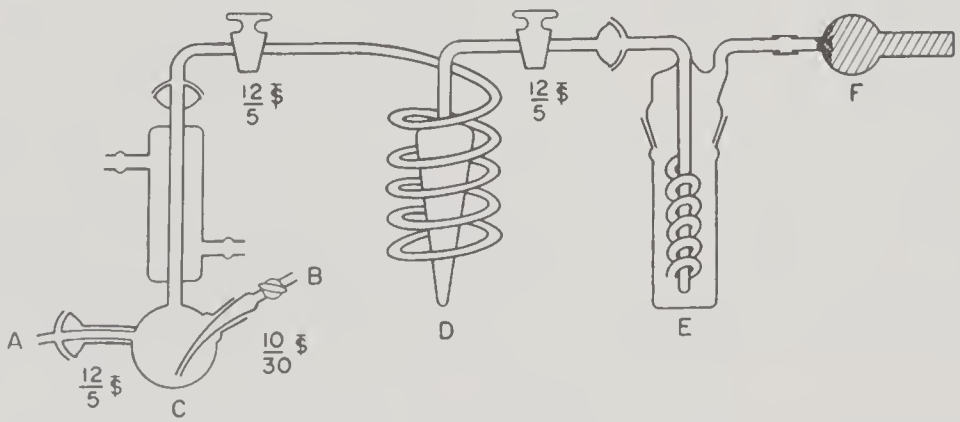


Fig. III, 3. Apparatus for the reductive alkylation of amines (W. Tarpey, H. Hauptmann, B. M. Tolbert and H. Rapoport). A, nitrogen inlet; B, microdropper; C, reaction flask (5 ml.); D, Dry Ice-cooled trap; E, alkali trap; F, soda lime tube.

fluxed for 4 hours at 100–105°, then cooled and treated with 0.9 ml. of 6 *N* hydrochloric acid. The exhaust end of the system is attached to a vacuum line, and the mixture is again warmed for 10 minutes under reflux. The two traps are removed (Note 2), and the mixture is evaporated to dryness under vacuum. The soft, yellow residue is dissolved in chloroform, and the filtered solution is evaporated under reduced pressure. The product is crystallized from acetone; yield 0.463 g., m.p. 187–188°. Second and third crops, totaling 0.455 g., are crystallized from a chloroform-ether mixture. The combined yield is 78%, and the molar specific activity is the same as that of the starting material (Note 3). Analysis by two-dimensional paper chromatography[1] (Note 4) and radioautography[2] shows the presence of only one radioactive compound.

B. Notes

1. The reaction employed is the Eschweiler-Clarke modification of the Leuckart reaction[3] for the reductive alkylation of amines by the action of amines by the action of aldehydes, or ketones, and formic acid.

2. Formaldehyde with considerable specific activity is recovered from the Dry Ice trap as the dimedon derivative (3 mg.). The carbon dioxide, recovered from the alkali trap as the barium salt (1.02 g., 5.2 mmoles), is virtually nonradioactive.

3. In a check reaction, using formaldehyde and formic-C^{14} acid, the product and recovered formaldehyde were nonradioactive, but the evolved carbon-C^{14} dioxide had the same molar specific activity as the starting formic-C^{14} acid. This data clarifies the role of formic acid in the Wallach alkylation as that of reducing the condensation product of amine and carbonyl compound.

4. Butanol-propionic acid is used in one direction, and phenol-water in the other. An isolation by countercurrent distribution and a sensitive microdetermination of Demerol are described by Burns.[4]

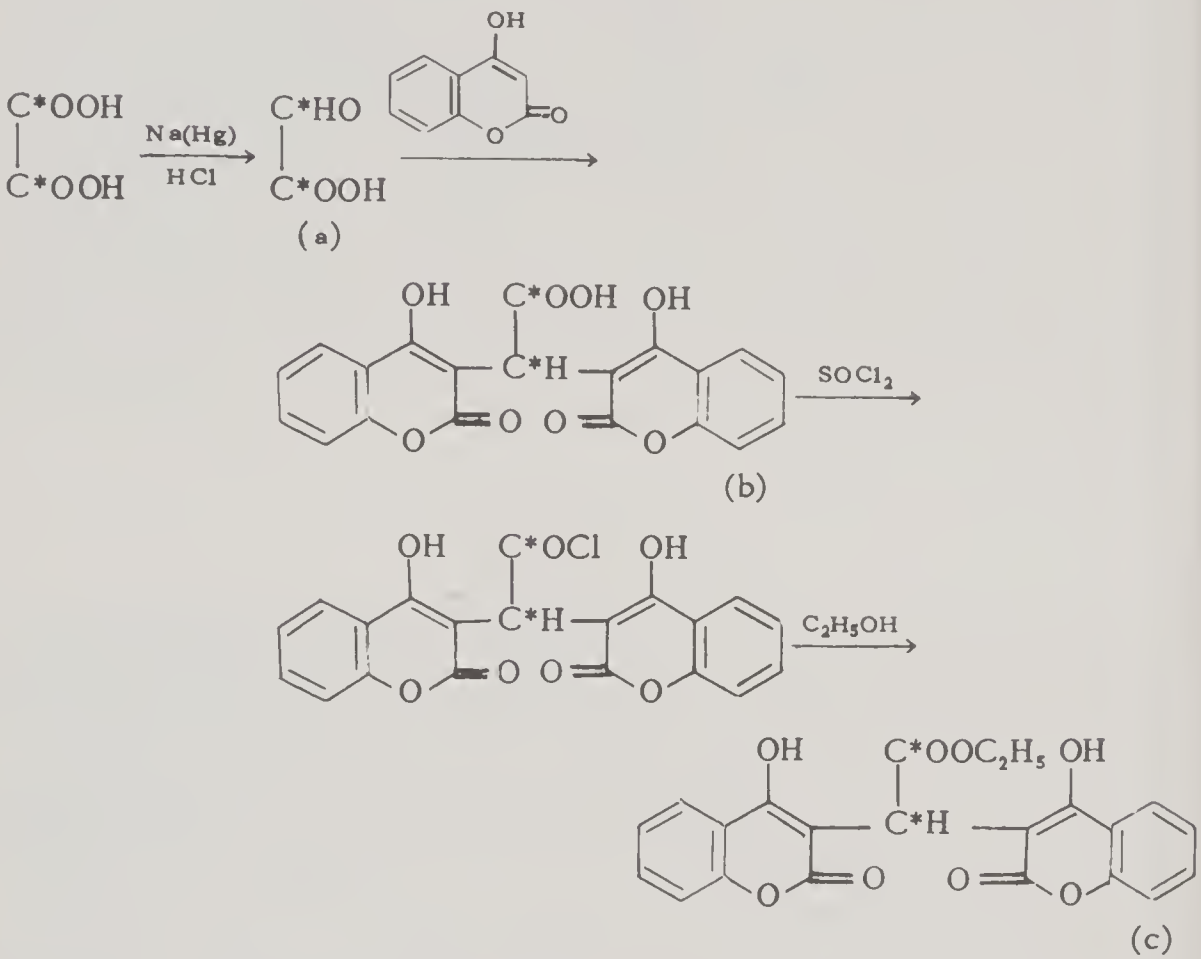
[1]A. A. Benson, J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas and W. Stepka, J. Am. Chem. Soc., *72*, 1710 (1950); C. E. Dent, Biochem. J., *43*, 169 (1948); *41*, 240 (1947); R. Consden, A. H. Gordon and A. J. P. Martin, Biochem. J., *41*, 590 (1950).

[2]R. M. Fink, C. E. Dent and K. Fink, Nature, *160*, 801 (1947); M. Calvin and A. A. Benson, Science, *109*, 140 (1949).

[3]E. Staple and E. C. Wagner, J. Org. Chem., *14*, 559 (1949); H. T. Clarke, H. B. Gillespie and S. Z. Weisshaus, J. Am. Chem. Soc., *55*, 4571 (1933).

[4]*Organic Reactions*, Vol. V. Wiley, New York, 1949, p. 301; J. J. Burns, B. L. Berger, P. A. Lief, A. Wollack, E. M. Papper and B. B. Brodie, J. Pharm. Exp. Therap., *114*, 289 (1955).

ETHYL BIS(4-HYDROXY-2-OXO-2*H*-1-BENZOPYRAN-3-YL)ACETATE-C_2^{14}
(C_2^{14}-Pelentan)



A. Murray, III, C. W. Bills and A. R. Ronzio, J. Am. Chem. Soc., *74*, 2405 (1952); Atomic Energy Commission Report, AECU-833; Nuc. Sci. Abstracts, *4*, 4246 (1950).

A. Procedure

(a) Glyoxylic-C_2^{14} Acid (Note 1). To a stirred solution of 0.150 g. of sodium oxalate-C_2^{14} (Note 2) in 20 ml. of water, cooled with a bath at 5° (Note 3), is added 20 g. of 1.2% sodium amalgam. The mixture is maintained within pH 1.5-2.0 by the dropwise addition of 6 *N* hydrochloric acid (Note 4). Additional amalgam (20–25 g.) is added, as needed, during the reduction period of 1 hour. The aqueous solution is separated from the mercury; yield 85–90% (Note 5).

(b) Bis(4-hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)acetic-C_2^{14} Acid, {Bis [3-(4-hydroxycoumarinyl)]acetic-C_2^{14} Acid} (Note 6). A fresh aqueous solution of glyoxylic-C_2^{14} acid prepared from 10.1 mmoles of sodium formate-C^{14} is added to a clear solution of 3.26 g. (20.1 mmoles) of 4-hydroxycoumarin in 465 ml. of water boiling under reflux (Note 7). The mixture is refluxed for 20 minutes and treated with 10 ml. of concentrated hydrochloric acid, and the heating is continued 10 minutes. The

precipitate is collected by filtering the hot mixture, then washed with 20 ml. of boiling 0.6 *N* hydrochloric acid and dried in a vacuum desiccator; yield 1.180 g. (Note 8).

(c) *Ethyl Bis(4-hydroxy-2-oxo-2H-1-benzopyran-3-yl)acetate-*C_2^{14}, {*Ethyl* Bis[3-(4-hydroxycoumarinyl)]acetate-C_2^{14}}. A stirred mixture of 0.5 g. of the crude acid and 7 ml. of purified thionyl chloride is refluxed at 80° for 20 minutes, then cooled and evaporated to dryness at reduced pressure (Note 9). The residual bis(4-hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)acetyl-C_2^{14} chloride is treated with 25 ml. of absolute ethanol, and the solution is refluxed for 4 hours. The solution is decolorized with carbon and evaporated with a stream of warm air, and the residue is dried over calcium chloride in a vacuum desiccator. The crude product, obtained in quanitative yield, is recrystallized from heptane; yield 86–90%, m.p. 150–152° (Note 10).

B. Notes

1. The procedure is an adaptation of that described by Mohrschulz.[1] The reduction with sodium amalgam was found to be more efficient than the electrolytic method of Tafel;[2] also see Bills and Ronzio.[3]

2. The alkaline melt from a sodium formate fusion may be used; see oxalic-C_2^{14} acid, reference 6.

3. The water-bath, containing a beaker (4 × 10 cm.) equipped with a glass-jacketed, magnetic stirring bar and the glass electrodes of a pH meter, is cooled by the periodic addition of ice.

4. The yield of glyoxylic acid drops to 63% at pH 2.5–3.0 and 37% at pH 3.5–4.0.

5. The concentration of solutions of glyoxylic acid may be estimated by determining the amount of insoluble condensation product formed in a slightly acid solution containing an excess of 4-hydroxycoumarin; see (b). The precipitation is about 82% efficient compared to the reducing action of the aldehyde on Tollen's reagent (weighing the metallic silver as the halide). No precipitate is formed with dimedon under acidic conditions. The phenylhydrazone decomposes at 144°.

6. The method is a modification of the method described by Sulliven[4] for condensing aldehydes with 4-hydroxycoumarins; also see Grüssner.[5]

7. The preparation of 4-hydroxycoumarin from methyl acetylsalicylate (22% yield) is described by Stahmann.[6] The concentration of the boiling solution is about 7 mg. per ml. of water.

8. The acid prepared by Sullivan[4] melted at 244–245°; dimethyl ether methyl ester, m.p. 160–161°. The acid prepared by Grüssner[5] melted at 234–236°.

9. The flask and condenser, protected with a tower filled with calcium chloride, are evacuated with an aspirator.

10. Purification by recrystallization from ethanol (97% recovery) is preferable for larger quantities. The product shows no melting point depression upon mixed melting with an authentic sample.

C. Other Preparations

The preparation of glyoxylic-C_2^{14} acid by reduction of oxalic-C_2^{14} acid with magnesium in the presence of mercuric chloride is described by Weinhouse;[7] yield 48% based on bisulfite binding.[8] The final yield of aqueous solution of purified product is 25–39%.

[1]W. Mohrschulz, Z. Electrochem., *32*, 434 (1926).

[2]J. Tafel and G. Friedrichs, Ber., *37*, 3187 (1904).

[3]C. W. Bills and A. R. Ronzio, Atomic Energy Commission Report, AECU-835; Nuc. Sci. Abstracts, *4*, 4248 (1950).

[4]W. R. Sullivan, C. F. Huebner, M. A. Stahmann and K. P. Link, J. Am. Chem. Soc., *65*, 2288 (1943).

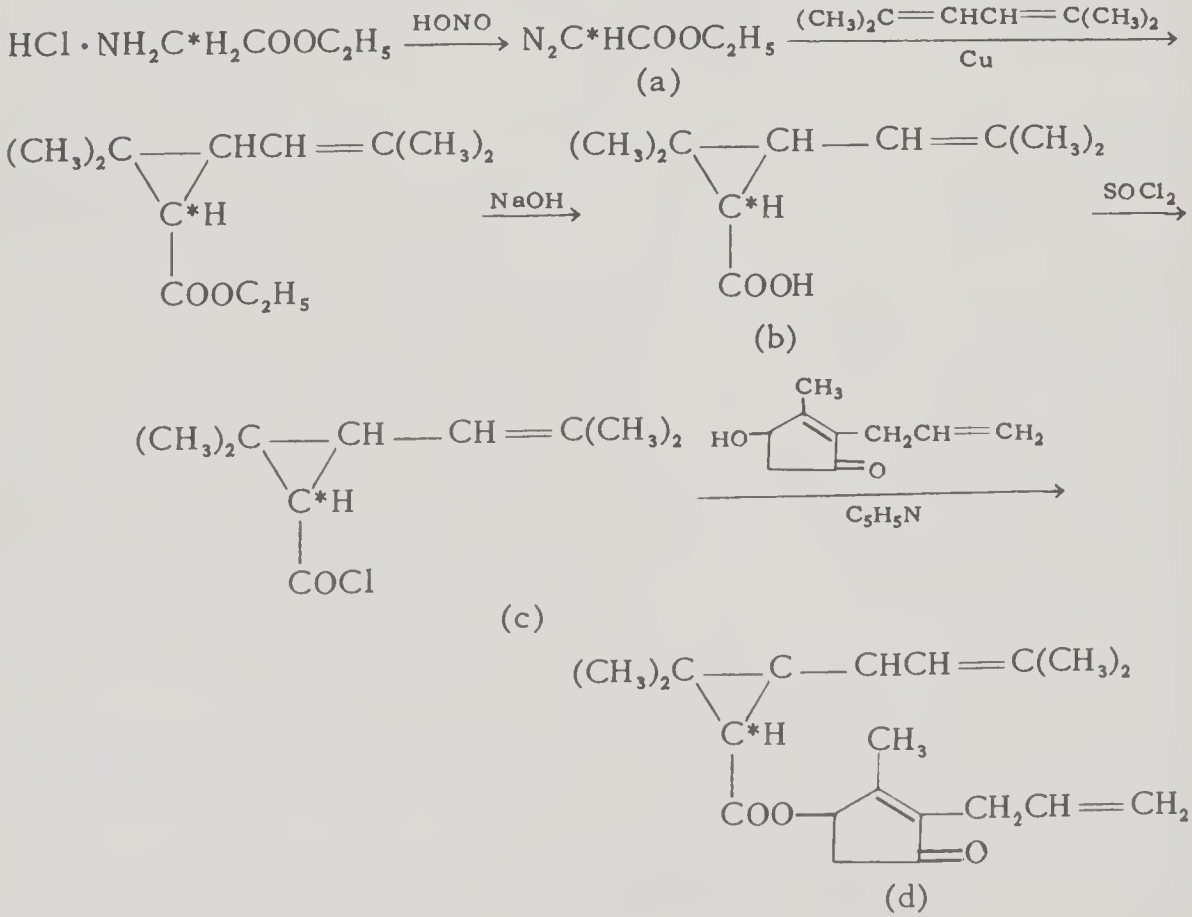
[5]A. Grüssner, Jubilee Vol. Emil Barell, *1946*, 238; Chem. Abstracts, *41*, 6232 (1947).

[6]M. A. Stahmann, I. Wolff and K. P. Link, J. Am. Chem. Soc., *65*, 2285 (1943).

[7]S. Weinhouse and B. Friedmann, J. Biol. Chem., *191*, 707 (1951).

[8]F. P. Clift and R. P. Cook, Biochem. J., *26*, 1788 (1932).

3-ALLYL-2-METHYL-4-OXO-2-CYCLOPENTEN-1-YL 2,2-DIMETHYL-3-(2-METHYLPROPENYL)CYCLOPROPANECARBOXYLATE-2-C^{14} (C_1^{14}-Allethrin)



F. Acree, Jr., C. C. Roan and F. H. Babers, J. Econ. Entomol., *47*, 1066 (1954).

A. Procedure

(a) *Ethyl Diazoacetate-2-C^{14}* (Note 1). The diazo ester is prepared batchwise by using two small separatory funnels, and controlling the temperature (exothermic) by swirling the funnel in a water-bath maintained at 15°. To a cooled mixture of 3.54 g. (25.5 mmoles) of glycine-2-C^{14} ethyl ester hydrochloride, 2.6 g. (37 mmoles) of sodium nitrite, 0.030 g. (0.22 mmole) of sodium acetate, 4 ml. of 2,5-dimethyl-2,4-hexadiene (Note 2) and 14 ml. of water is added dropwise 0.5 ml. of 10% sulfuric acid. The hexadiene layer is thoroughly equilibrated, separated and passed with mild suction through a column containing 5g. of anhydrous sodium carbonate. The aqueous layer is submitted to four more cycles with the same amounts of extraction solvent and acid (Note 3).

(b) *2,2-Dimethyl-3-(2-methylpropenyl)cyclopropanecarboxylic-2-C^{14} Acid, (C_1^{14}-Chrysanthemummonocarboxylic Acid)* (Note 4). The combined hexadiene extract is added dropwise to a refluxing mixture of 10 ml. of 2,5-dimethyl-2,4-hexadiene and 0.300 g. of copper powder (Note 5). The excess 2,5-dimethyl-2,4-hexadiene is distilled at 30–35° (12 mm.) (Note 6). The residual ethyl 2,2-dimethyl-3-(2-methylpropenyl)cyclopropanecarboxylate-2-C^{14} (Note 7) is refluxed for 2 hours with a solution of 2 g. of sodium hydroxide and 3 ml. of water in 15 ml. of alcohol. The alcohol is evaporated, and the residue (3.16 g.) is diluted with water and washed with ether (Note 8). The product is isolated by acidification, ether extraction and distillation; yield 2.26 g. (49.8% based on glycine-2-C^{14}), b.p. 107–109° (1 mm.) (Note 9).

(c) *3-Allyl-2-methyl-4-oxo-2-cyclopenten-1-yl 2,2-Dimethyl-3-(2-methylpropenyl)cyclopropanecarboxylate-2-C^{14}, (Allethronyl C_1^{14}-Chrysanthemummonocarboxylate)* (Note 10). The chrysanthemummonocarboxylic acid (0.100 g., 0.59 mmole) is treated with 0.100 g. (0.89 mmole) of purified thionyl chloride in 1.5 ml. of dry Skellysolve B under anhydrous conditions. The mixture is stored overnight and evaporated at 12 mm. The C_1^{14}-chrysanthemummonocarbonyl chloride is distilled with a bath at 95–100° (1 mm.) into a trap.

A solution of the acid chloride in 0.5 ml. of dry benzene is treated with 0.100 g. (0.72 mmole) of 2-allyl-4-hydroxy-3-methyl-2-cyclopenten-1-one (Note 11) and 0.075 g. (0.95 mmole) of dry pyridine in 1 ml. of benzene. The mixture is stored overnight, transferred to a centrifuge tube with 1 ml. of dilute hydrochloric acid and 1.5 ml. of Skellysolve B in small portions, and washed with water by centrifuging and use of a pipette. The crude product is evaporated at 35–40° (1 mm.); yield 0.1674 g., 93% (Note 12). Purification is accomplished by partition chromatography between hexane and nitromethane on a column of silicic acid under air pressure (3–4 p.s.i.). The eluate is collected in 5-ml. portions near the expected break between fractions (Note 13) and evap-

orated, the product occurring at 131–250 ml.; yield 0.1329 g., 74.5% based on the acid.

B. Notes

1. The procedure is an adaptation of Laforge's[1] improvement of that of Womack and Nelson.[2] Ethyl diazoacetate is rapidly decomposed by acid, and distillation is dangerous, for the compound is explosive. The product should be used as soon as possible. The extraction solvent is the reagent used in the next step, rather than ether.

2. Directions are given by Campbell[3] for preparing the solvent by catalytic isomerization of 2,5-dimethyl-1,5-hexadiene.[4]

3. The final aqueous solution contains 1.2% of the initial radioactivity.

4. The procedure is an adaptation of that of Campbell.[3]

5. The yield of nitrogen is about 97%.

6. The distillate contains only a trace of radioactivity.

7. Ethyl *cis,trans*-chrysanthemummonocarboxylate boils at 95–115° (12 mm.).

8. The nonacidic products contain 8.3% of the radioactivity of the glycine ester hydrochloride.

9. The combined forerun and residue (0.8 g.) possess 20.7% of the radioactivity of the glycine ester hydrochloride. The mixed acid crystallizes upon cooling. The *cis*-acid, recrystallized from ethyl acetate, melts at 113–116°; the *trans*-acid melts at 54°.[3]

10. The procedure is an adaptation of that of Schecter;[5] see C_1^{14}-*trans*-allethrin.

11. Allethrolone is purified by distillation, after regeneration from the recrystallized semicarbazone.

12. The radioactivity of the combined distillate, residue and washes is equal to 8% of that of the acid.

13. Chrysanthemummonocarboxylic acid and anhydride fractions (56–125 ml.) precede the product from the column, while the allethrolone and acid chloride remain on the column. Only 91.4% of total products are recovered from the column, even by the use of acetone as eluant.

[1]F. B. Laforge, W. A. Gersdorff, N. Green and M. S. Schechter, J. Org. Chem., *17*, 381 (1952).

[2]*Organic Syntheses*, Coll. Vol. III, Wiley, New York, 1955, p. 392.

[3]I. G. M. Campbell and S. H. Harper, J. Chem. Soc., *1945*, 283.

[4]A. L. Henne, H. Chanan and A. Turk, J. Am. Chem. Soc., *63*, 3474 (1941).

[5]M. S. Schechter, N. Green and F. B. Laforge, J. Am. Chem. Soc., *71*, 3165 (1949).

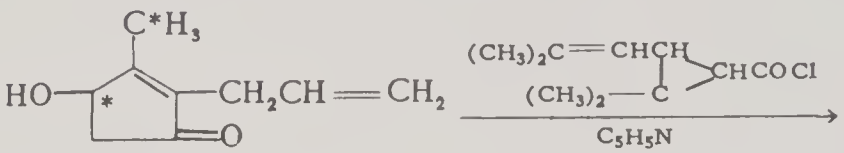
3-ALLYL-2-METHYL-C^{14}-4-OXO-2-CYCLOPENTEN-1-YL-1-C^{14} *trans*-CHRYSANTHEMUMMONOCARBOXYLATE (C_1^{14}-*trans*-Allethrin)

$$(C^*H_3)_2CO \xrightarrow{SeO_2} C^*H_3COC^*HO \xrightarrow[NaOH]{CH_2{=}CHCH_2CH_2COCH_2COOH}$$

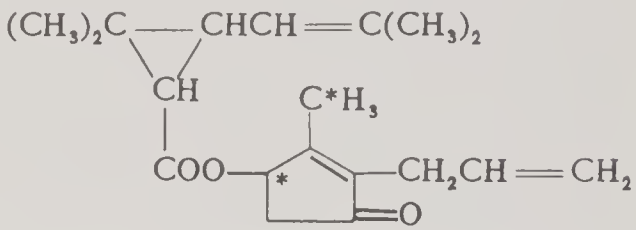
(a)

$$CH_2{=}CHCH_2CH_2COCH_2C^*HCOC^*H_3 \xrightarrow{NaOH}$$

(b)



(c)



(d)

E. P. W. Winteringham, A. Harrison and P. M. Bridges, Biochem. J., *61*, 359 (1955).

A. Procedure

(a) *Pyruvaldehyde-1,3-C_2^{14}.* To a solution of 3.8 g. of purified selenium dioxide in 20 ml. of redistilled, peroxide-free dioxane and 1.4 ml. of water at 50° is added 2.5 ml. of 2-propanone-1,3-C_2^{14}. The mixture is refluxed for 4 hours and distilled in nitrogen. The distillate is evaporated at 0° under reduced pressure; yield of clear syrup 40% (Note 1).

(b) *3-Hydroxy-8-nonene-2,5-dione-1,3-C_2^{14}* (Note 2). Ethyl 3-oxo-6-heptenoate[1] (2.27 ml.) is shaken at room temperature for 72 hours with 9.3 ml. of 10% potassium hydroxide (Note 3). Any insoluble oil is removed, the pyruvaldehyde-1,3-C_2^{14} is added with water, and the mixture is adjusted to pH 8.0 and stored at 35° for 6–7 hours until the formation of an oily phase is complete (Note 4). The mixture is saturated with sodium chloride, and the product is extracted into ether solution, which is dried over sodium sulfate and concentrated. The product is collected by fractional distillation (0.15 mm.), b.p. 89–95° (Note 5).

(c) *2-Allyl-4-hydroxy-3-methyl-C^{14}-2-cyclopenten-1-one-4-C^{14}, (C_1^{14}-Allethrolone)* (Note 2). 3-Hydroxy-8-nonene-2,5-dione (32 g.) is cyclized by shaking for 1.5 hours with 220 ml. of 10% sodium hydroxide under nitrogen. The mixture is acidified with hydrochloric acid (1:3), then saturated with sodium chloride and extracted with ether. The extract is dried over sodium sulfate and concentrated, and the product is fractionally distilled (0.1 mm.), b.p. 100–109° (Note 6).

(d) *3-Allyl-2-methyl-C^{14}-4-oxo-2-cyclopenten-1-yl-1-C^{14}* trans-*Chrysanthemummonocarboxylate.* The 2-allyl-4-hydroxy-3-methyl-C^{14}-2-cyclopenten-1-one-4-C^{14} is esterified in benzene solution, containing 2 equivalents of pyridine and a slight excess of *trans*-chrysanthemummonocarbonyl chloride[1] (Note 7), by storage overnight at room temperature. The solution is washed with saturated aqueous sodium bicarbonate and water, then dried over sodium sulfate and evaporated at a water pump at 100°. The product is collected by fractional distillation (Note 8); yield 0.441 g., purity 47% (Note 9). Purification is accomplished by reversed-phase ascending paper chromatography[2] in all-glass columns under reduced pressure[3] at 25°, up to 1 mg. of crude product being applied to single 1 1/8 inch paper strips in ether solution (Note 10). The chromatogram is developed with 45% ethanol-50% water – 5% ammonium hydroxide (sp. gr. 0.90) by volume (Note 11), and the product zone, R_f 0.40, is eluted with 95% alcohol at 5°.

B. Notes

1. The yield is based on the titration of trial preparations with alkali in the presence of hydrogen peroxide.[4] Pyruvaldehyde is hygroscopic and polymerizes very readily, $n_D^{17.5}$ 1.4002.

2. The procedure is a one-twenty-fifth scale-down of that here described by Crombie.[5] The method is that of Schechter[6] for preparation of the ester intermediates. Oxidative losses during ether extraction are reduced by using peroxide-free ether containing 0.01% hydroquinone. At the reduced scale, yields become slightly lower, and the fraction cutting is made less rigorous in the vacuum microdistillations.

3. Potassium hydroxide gives less deeply colored solutions than sodium hydroxide.

4. Up to 48 hours is required at 20°.

5. 3-Hydroxy-8-nonene-2,5-dione, b.p. 85–90° (0.07 mm.), n_D^{25} 1.4657, 58% yield.[6] The carbon dioxide evolved in this reaction is nonisotopic.

6. 2-Allyl-4-hydroxy-3-methyl-2-cyclopenten-1-one, b.p. 100–103° (0.15 mm.), n_D^{25} 1.5141, 59% yield; semicarbazone, m.p. 213–214° (dec.); 3,5-dinitrobenzoate, m.p. 129–130°.[6]

7. *trans*-Chrysanthemummonocarbonylchloride (67%), b.p. 96.0–96.5° (12 mm.), n_D^{20} 1.4852, is freshly prepared by reaction of the acid[1,7] with thionyl chloride in light petroleum at room temperature; see C_1^{14}-allethrin.

8. The alkenylrethrins are stable to distillation. Allethrin boils at 127–130° (2μ), n_D^{20} 1.5051–1.5056; yield 47% based on the acid chloride.

9. The purity is based on radio-assay of a paper chromatogram.[8] A 4π beta counter for scanning paper chromatograms (32% effective efficiency for C^{14}) is described by Harrison.[9] A bio-assay is described by Hoskins.[10]

10. Acetone interferes with some separations. Whatman No. 1 paper is soaked for 30 minutes in a solution containing (by volume) 45% ethanol, 50% water and 5% concentrated hydrochloric acid, then is soaked in dilute aqueous ammonia and water, and finally drained and dried. The paper is dipped once in a 3% (w/v) solution of USP petroleum jelly in ether, drained and dried.

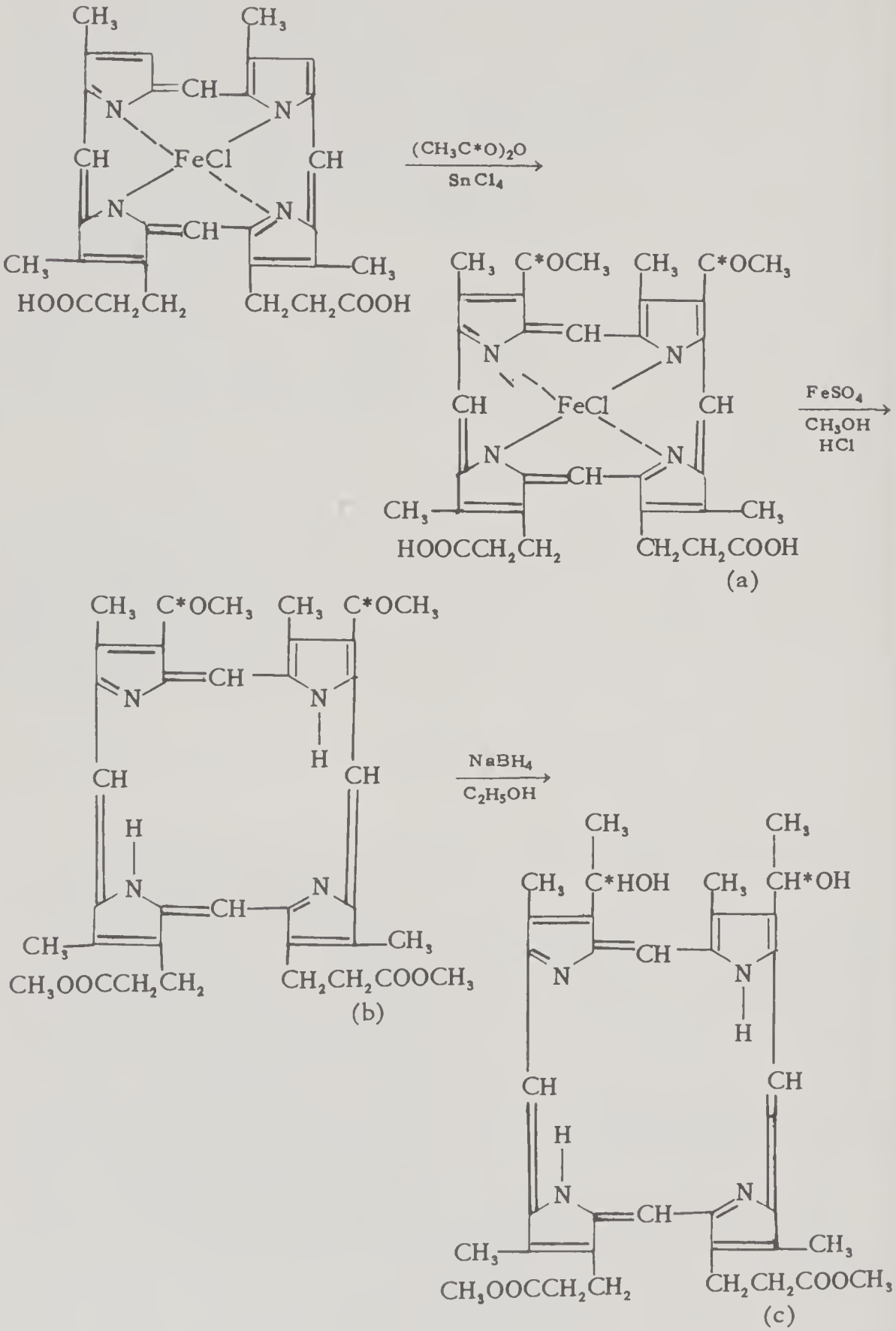
Purification by column chromatography is described by Acree; see *cis*, *trans*-C_1^{14}-allethrin.

11. A test sufficiently sensitive to detect less than 1 μg. of pyrethroids, or their unsaturated derivatives, in a reference chromatogram consists of dipping the dry paper once in 0.1% aqueous, neutral potassium permanganate and immediately washing-free of excess permanganate with distilled water. A blue zone is developed by dipping the almost dry paper in a 0.5% (w/v) solution of benzidine in dry acetone containing 5% (v/v) glacial acetic acid.

Another method for determining allethrin by reaction with ethylenediamine is described by Hogsett.[11]

[1]H. J. Sanders and A. W. Taff, Ind. Eng. Chem., *46*, 414 (1954).
[2]F. P. W. Winteringham, Science, *116*, 452 (1952).
[3]F. P. W. Winteringham, P. M. Bridges and G. C. Hellyer, Biochem. J., *59*, 13 (1955).
[4]T. E. Friedmann, J. Biol. Chem., *73*, 331 (1927).
[5]L. Crombie, A. J. B. Edgar, S. H. Harper, M. W. Lowe and D. Thompson, J. Chem. Soc., *1950*, 3552.
[6]M. S. Schechter, N. Green and F. B. Laforge, J. Am. Chem. Soc., *71*, 3165 (1949).
[7]I. G. M. Campbell and S. H. Harper, J. Chem. Soc., *1945*, 283.
[8]F. P. W. Winteringham, A. Harrison and R. G. Bridges, Nucleonics, *10* (3), 52 (1952); Analyst, 77, 19 (1952).
[9]A. Harrison and F. P. W. Winteringham, Nucleonics, *13* (3), 64 (1955).
[10]W. M. Hoskins and P. S. Messenger, *Advances in Chemistry*, *1*, 93 (1950).
[11]J. N. Hogsett, W. Kacy and J. R. Johnson, Anal. Chem., *25*, 1207 (1953).

C_2^{14}-HEMATOPORPHYRIN DIMETHYL ESTER



A. K. Bruce, Atomic Energy Commission Report, UR-339; Nuc. Sci. Abstracts, 8, 5540 (1954).

A. Procedure

(a) *2,4-Bis(acetyl-1-C^{14})deuterohemin* (Note 1). A solution of 0.300 g. of deuterohemin (Note 2) and 1.5 ml. of bis(acetic-1-C^{14}) anhydride (Note 3) is stirred for 30 minutes, then cooled with an ice-bath and treated with 0.05 ml. of stannic chloride. The ice-bath is removed for 10 minutes; then the stirred solution again is cooled and treated with 0.05 ml. of stannic chloride. The mixture is stirred for 10 minutes at room temperature, then poured onto ice in a centrifuge cone and stored overnight in a refrigerator. The precipitate is collected, washed with water, dried under vacuum and dissolved in glacial acetic acid. The solution is diluted with ether and ligroin and cooled for 1 hour, and the precipitated crude product is collected and dried (Note 4).

(b) *Bis(acetyl-1-C^{14})deuteroporphyrin Dimethyl Ester* (Note 5). A solution of the crude 2,4-bis(acetyl-1-C^{14})deuterohemin, in methanol containing ferrous sulfate (3–5 g./100 ml.), is saturated with anhydrous hydrogen chloride and stored overnight. The solution is diluted with water, and the product is extracted into chloroform solution, which is washed with water, 5% ammonium hydroxide and water, then evaporated under vacuum. The residue is dissolved in chloroform and placed on a column of calcium carbonate, wet and developed with chloroform-benzene 1:15 (v/v), an ultraviolet lamp being used to determine the zones. The bands are extruded, eluted with chloroform and characterized by determining the ultraviolet absorption curves in chloroform (Note 6) and the specific activities. Analysis by paper chromatography indicates that each band is homogeneous (Note 7).

The separated monoacetyl-1-C^{14}-deuteroporphyrin dimethyl ester is converted to monoacetyl-1-C^{14}-deuterohemin by the method of Fischer and Pützer[1] as follows. To a solution of the monoacetyl ester (band 2) in saturated boiling acetic acid-sodium chloride is added dropwise a boiling solution of ferrous acetate until the color changes from red to brown and the ultraviolet fluorescence disappears (40 drops). The solution is stored for several hours; then the product is extracted into chloroform and acetone, and the solution is washed with water and evaporated under vacuum. The monoacetyl-1-C^{14}-deuterohemin is dissolved in 30 ml. of acetic anhydride and resubmitted to the acetylation and esterification reactions. The specific activity of the acetyl(acetyl-1-C^{14})deuteroporphyrin dimethyl ester is 55% of that of the bis(acetyl-1-C^{14}) product (Note 8).

The combined crude product is purified by chromatography on calcium carbonate; yield 0.034 g. (11.4% over-all).

(c) *Methyl 1,3,5,8-Tetramethyl-2,4-bis(1-hydroxyethyl-1-C^{14})porphyrin-6,7-dipropionate, (C_2^{14}-Hematoporphyrin Dimethyl Ester).* To a stirred solution of 0.034 g. of purified bis(acetyl-1-C^{14})deuteroporphyrin dimethyl ester in 40 ml. of hot ethanol is added sodium borohydride in

small portions at 5-minute intervals (Note 9). When the absorption spectrum changes to that of hematoporphyrin (20 minutes), the solvent is distilled under vacuum, and the crude product is extracted into chloroform, in which sodium borohydride is insoluble. The filtered solution is concentrated and placed on a sugar column, wet and developed with chloroform-benzene 1 : 14 (v/v). The fastest moving of the four components is further fractionated into four components on a column of calcium carbonate, using the same solvent. The product is identified by comparing the absorption spectra and paper chromatograms of the separated reduction products with an authentic reference sample (Note 10). The yield is 0.0052 g., and the molar specific activity is in agreement with that of the diacetyldeuteroporphyrin dimethyl ester.

B. Notes

1. The procedure is an adaptation of the classical method of Fischer.[2] The volume of labeled acetic anhydride is kept as small as possible, and the recovered labeled mono-acetylated product then is reacted with a large excess of nonisotopic acetic anhydride.

2. Directions are given by Bruce for preparing the starting material by degradation[3] of protohemin by fusion with resorcinol. Analysis by column chromatography and ultraviolet spectrophotometry shows the presence of two major hemin components. No separation was attempted, because of the limited capacity of the silica gel columns.

3. Attempts to find an inert solvent for the reaction were unsuccessful, as were attempts to effect the condensation with acetyl chloride.

4. The crude product consists of both mono- and diacetylated condensation products.

5. The method is that of Grinstein.[4]

6. The absorption maxima (mμ) and ratios of intensities (in parentheses) are in good agreement with the values determined by Lemberg[5] for monoacetyldeuteroporphyrin dimethyl ester (band 2) and diacetyldeuteroporphyrin dimethyl ester (band 4):

Band	I	II	III	IV
2	634(0.164)	577(0.817)	548(1.150)	509(1.000)
4	641(0.268)	587(0.628)	551(0.718)	518(1.000)

7. The chromatogram is developed by a modification of the method of Chu,[6] employing decane-propanol 10 : 1 (v/v) by the descending technique.

8. On the basis of absorption spectrum and R_f value (0.35), the material is indistinguishable from (b).

9. The reagent is specific for the reduction of aldehydes, ketones and acid halides; acids, esters and anhydrides being unaffected. Poor yields are obtained with ether or pyridine as solvent.

10. The paper chromatogram[7] is developed with decane-dioxane 1 : 1 (v/v) on Whatman No. 1 filter paper by the descending technique (R_f 0.72). The absorption maxima (mμ) and ratios of intensities (in parentheses) for hematoporphyrin dimethyl ester are:

I 624(0.235), II 570(0.500), III 535(0.650), and IV 501(1.000).

Pure hematoporphyrin dimethyl ester melts at 212°. A porphyrin degradation is described by Shemin.[8]

[1]H. Fischer and B. Pützer, Hoppe-Seyler's Z. physiol. Chem., *154*, 39 (1926).
[2]H. Fischer and H. Orth, *Die Chemie des Pyrrols*, Band II, 1, Hälfte, Leipzig, Adademische Verlagsgesellschaft M.B.H. (1937), p. 305.
[3]*Idem.*, p. 414.
[4]M. Grinstein, J. Biol. Chem., *167*, 515 (1947).
[5]R. Lemberg and J. E. Falk, Biochem. J., *49*, 674 (1951).
[6]T. C. Chu, A. A. Green and E. J. Chu, J. Biol. Chem., *190*, 643 (1951); T. C. Chu and E. J. Chu, J. Biol. Chem., *208*, 537 (1954); *212*, 1 (1955).
[7]J. E. Falk and A. Benson, Biochem. J., *55*, 101 (1953).
[8]D. Shemin and J. Wittenberg, J. Biol. Chem., *192*, 315 (1951).

ACETYL-1-C^{13} DIHYDROGEN PHOSPHATE

$$CH_3C^*OCl \xrightarrow{AgH_2PO_4} CH_3C^*OOP(OH)_2 \xrightarrow{AgNO_3} CH_3C^*OOP(OAg)_2$$

H. J. Strecker, H. G. Wood and L. O. Krampitz, J. Biol. Chem., *182*, 525 (1950).

A. Procedure

Acetyl-1-C^{13} dihydrogen phosphate is prepared from acetyl-1-C^{13} chloride by the procedure of Lipmann and Tuttle,[1] which follows.

A solution of 17 ml. (0.24 mole of acetyl chloride in an equal volume of ether is added dropwise, over a period of 10 minutes with shaking, to an ice-cooled, dry ethereal suspension of silver dihydrogen phosphate (Note 1). The clumps of silver chloride are broken up by vigorous shaking at room temperature for 10 minutes. The pH is adjusted to 3.0–3.5 with the addition of 120–140 ml. of chilled 2 *N* sodium carbonate in portions (Note 2). The ether and aqueous layers are filtered through Celite to remove silver chloride, then the acetic acid (Note 3) is removed

by two 2-minute extractions in the cold (Note 4) with three times the volume of ether. The cold aqueous solution is neutralized to the phenol red end point (20–22 ml. of 33% sodium hydroxide solution), slowly and with constant shaking to avoid local over-alkalinization (Note 5). The solution is frozen for at least 1 hour at −35° (Note 6) to complete crystallization of the sodium phosphate, which is removed by breaking up the frozen mass, pressing it on a Büchner funnel and sucking dry as it thaws slowly (Note 7). The filtrate is adjusted to pH 7–8, a 10% excess of silver nitrate (Note 8) is added (10–25 ml. of 25% solution), the pH is readjusted, and the precipitated chloride and inorganic phosphate are filtered off with the aid of Celite (Note 9). Disilver acetylphosphate is precipitated with the addition of twice the stoichiometric amount of silver nitrate (about 120 ml.). The copious precipitate is collected, washed with a little ice water, two portions of 33% alcohol, alcohol and with ether, then dried under vacuum over phosphorus pentoxide (Note 10); yield 13–16 g. (Note 11). Homologous acylphosphates are prepared in the same manner.

B. Notes

1. Although no silver phosphate of this composition is known as a well defined compound, a mixture of 1 mole of trisilver phosphate and 2 moles of phosphoric acid reacts in the desired manner. In a 250-ml. centrifuge bottle are mixed 32 g. (76 mmoles, 230 milliequivalents) of silver phosphate and 11 ml. (176 mmoles) of 90% phosphoric acid. Heat is evolved, the bright yellow color changes to a gray, and the syrupy mixture largely solidifies on cooling. The mass is finely distributed in 20–25 ml. of ether, giving a homogeneous light gray suspension.

2. The ether layer prevents excessive foaming.

3. At this pH the acetic acid is largely in the nonionized, easily extractable form. Its removal prevents contamination with silver acetate.

4. The pH-decomposition curve[1] for acetyl dihydrogen phosphate indicates that only slight decomposition occurs in the cold solution. Between 70% and 50% of the total phosphorus is acetyl bound at this stage.

5. The stability of acetyl dihydrogen phosphate rapidly diminishes on either side of pH 5 to 6.

6. Frozen solutions of acetyl dihydrogen phosphate may be kept for weeks without appreciable decomposition.

7. The crystal mass should not rise above −5°. Analysis shows that almost no acetylphosphate remains on the filter.

8. The amount of silver used is based upon an analysis of an aliquot of the filtrate for chloride and phosphate, the silver salts of which are much less soluble than is acetyl disilver phosphate.[2]

9. The addition of a few drops of silver nitrate solution should produce a precipitate which dissolves readily upon shaking, forming a bluish white cloud.

10. Dry acetyl disilver phosphate is stable for years in the dark. The product may be analyzed[1] by acid hydrolysis and alkaline titration of the steam-distilled acetic acid formed, by precipitation of phosphate, or by a colorimetric determination of acetyl-bound phosphate.

11. If the product is not crystalline, the crude salt is dissolved with slightly less than an equivalent of 0.25 *M* sodium chloride solution; then the solution is tested for the presence of inorganic phosphate by dropwise addition of silver nitrate solution. Any yellowish precipitate is discarded, and the isolation then is carried out as described.

[1]F. Lipmann and L. C. Tuttle, J. Biol. Chem., *153*, 571 (1944).
[2]F. Lynen, Ber., *73B*, 367 (1940).

2-HYDROXYPROPYL-2-C^{14} DIHYDROGEN PHOSPHATE

$$CH_3C^*OCONH_2 \xrightarrow{LiAlH_4} CH_3C^*HOHCH_2NH_2 \xrightarrow[\text{2. } Ba(OAc)_2 \text{ 3. Versene}]{\text{1. } POCl_3} CH_3C^*HOHCH_2O\overset{\overset{O}{\|}}{P}(OH)_2$$

D. P. Groth and G. A. LePage, J. Am. Chem. Soc., 77, 1681 (1955).

A. Procedure (Note 1)

To 3 ml. of a stirred 0.5 *M* ether solution of lithium aluminum hydride[1] is added a solution of 0.022 g. of pyruvamide-2-C^{14} in 10 ml. of absolute ether at a rate to maintain gentle reflux. The mixture is refluxed for 4 hours, stored overnight, concentrated to 3–5 ml., then treated with 10 ml. of anhydrous chloroform, and evaporation is repeated. The mixture is refluxed for 4–5 hours with 0.6–0.7 ml. of phosphorus oxychloride in 10 ml. of chloroform, then stored overnight and diluted with 15 ml. of water. The chloroform is evaporated on a steam-bath, and the aqueous solution is heated at 100° for 2 hours (Note 2), then cooled and adjusted to pH 7 with 10 *N* potassium hydroxide. The stirred solution is heated for 5 minutes at 100° with 12–14 ml. of 1 *M* barium acetate (Note 3). The barium phosphate is separated by centrifuging and washed with five 25-ml. portions of water (Note 4). The combined solution is percolated through a 2 × 20-cm. column of Dowex 50 (H form) which is washed with five 10-ml. portions of water. The combined eluate is concentrated to 5 ml. under vacuum at room temperature (Note 5), and the product is purified by two-dimensional descending paper chromatography (Note 6),

using the solvent systems: isopropyl alcohol, concentrated ammonia, saturated aqueous versene solution (6:2:2); isopropyl alcohol, saturated aqueous versene solution, and 2 *M* chloroacetic acid (7:2:1). The overall yield is 25–39% based on pyruvamide (Note 7).

B. Notes

1. The method is a modification of that described by Lindberg.[2]

2. The aqueous solution, 1.3–1.5 *N* in hydrochloric acid, is heated to hydrolyze possible contaminating phosphate esters, the desired product being unaffected.

3. The product is not precipitated.

4. The precipitate is resuspended and heated with each wash.

5. The yield is 65–70% based on a determination[3] of organic phosphorus.

6. Aliquots (30 μmoles of organic phosphorus) are streaked along the top edge of 22½ × 18-inch sheets of Schleicher and Schüll No. 589 filter paper and dried. The product agrees in migration rate with authentic samples and is eluted from the appropriate section of paper with water.

7. The yield from the two chromatographic procedures is 77–92% and 50–61%, respectively. Once crystallized, "propanediol phosphate" is easily recrystallized.[2]

[1] *Organic Reactions*, Vol. VI, Wiley, New York, 1951, Chapter 10.

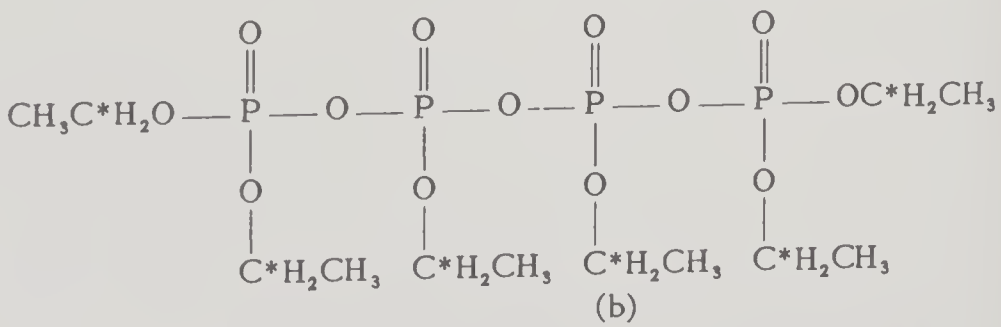
[2] O. Lindberg, Arkiv Kemi, Mineral. Geol., *21B* (3), 1 (1945); Chem. Abstracts, *40*, 6599 (1946); *23A* (2), 1, (1946); Chem. Abstracts, *41*, 1289 (1947).

[3] W. W. Umbreit, R. H. Burris and J. F. Stauffer, *Manometric Techniques* and *Tissue Metabolism*, Burgess Pub. Co., Minneapolis, Minn., 1945, p. 160.

HEXAKIS(ETHYL-1-C^{14}) TETRAPHOSPHATE

$$CH_3C^*H_2I \xrightarrow{Ag_3PO_4} OP(OC^*H_2CH_3)_3 \xrightarrow{P_2O_5}$$

(a)



(b)

R. W. Brauer and R. L. Pessotti, Science, *110*, 395 (1949); R. W. Brauer, J. Pharmacol. Exp. Therap., *92*, 162 (1948).

Procedure

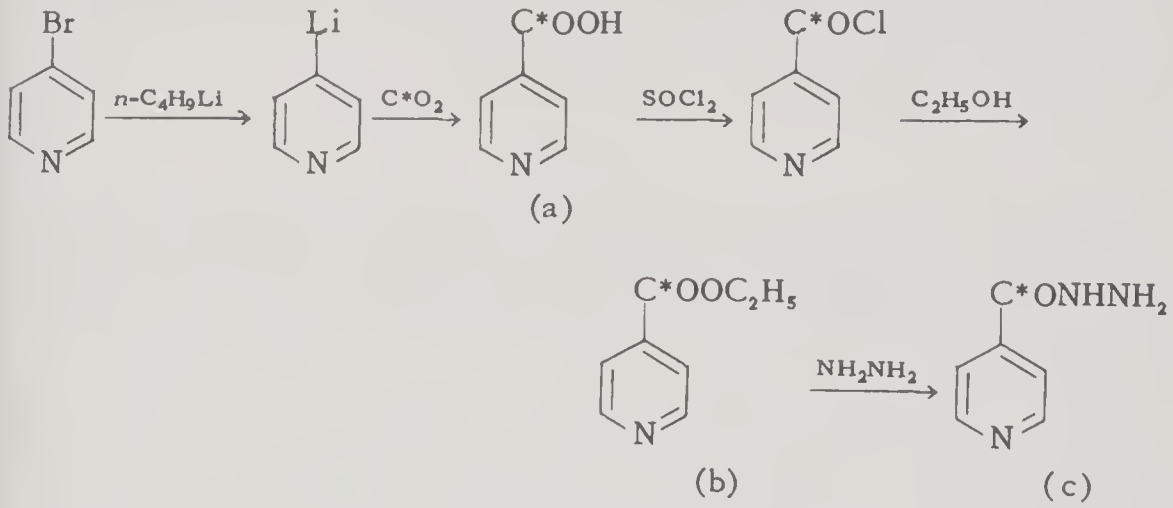
(a) *Ethyl-1-C^{14} Phosphate.* Silver phosphate is reacted with 3 moles of ethyl-1-C^{14} iodide by a suitable adaptation of the method of Baldwin and Higgins;[1] see butyl phosphate-P^{32}. Ethyl phosphate[2] boils at 215° (96–101° at 3–5 mm.), n_D^{25} 1.4039.

(b) *Hexakis(ethyl-1-C^{14})Tetraphosphate.* Phosphorus pentoxide (1 mole) is reacted with 2 moles of ethyl-1-C^{14} phosphate at 55° for 2 hours, then at 100° for 1 hour. The product[2] decomposes at 145–140°, d_4^{25} 1.280, n_D 1.2425.

[1]W. H. Baldwin and C. E. Higgins, J. Am. Chem. Soc., *74*, 2431 (1952).

[2]W. H. Woodstock, U. S. 2,402,703; Chem. Abstracts, *40*, 5444 (1946); H. Adler and W. H. Woodstock, Chem. Inds., *51*, 516 (1942).

ISONICOTINIC-C^{14} ACID HYDRAZIDE
(C_1^{14}-Isoniazid)



A. Murray III and W. H. Langham, J. Am. Chem. Soc., *74*, 6289 (1952).

A. Procedure

(a) *Isonicotinic-C^{14} Acid* (Note 1). To a stirred solution of 5.12 mmoles of butyllithium in 25 ml. of absolute ether, maintained at –78° under nitrogen, is added a solution of 7.68 mmoles of 4-bromopyridine in 30 ml. of dry ether at –50° to –40° (Note 2). After 2 minutes, the mixture is frozen with liquid nitrogen, evacuated, thawed with a Dry Ice-acetone-bath and carbonated with the carbon-C^{14} dioxide from 0.5064 g. (2.56 mmoles) of barium carbonate-C^{14}. The cold reaction mixture, after

hydrolysis with 10 ml. of 6 *N* hydrochloric acid, is made strongly basic with sodium hydroxide and continuously extracted with ether for 24 hours, then strongly acidified and extracted for 4 hours. The aqueous solution is adjusted to pH 3, and the product is collected by ether extraction for 4–5 days; yield 0.303 g., 96.0% (Note 3).

(b) *Ethyl Isonicotinate-C^{14}* (Note 4). A mixture of the isonicotinic-C^{14} acid and 2 ml. of purified thionyl chloride is refluxed for 15 minutes, then evaporated under reduced pressure. The residual solid, isonicotinoyl-C^{14} chloride hydrochloride, is refluxed for 1 hour with 4 ml. of absolute alcohol; then the solution is evaporated under vacuum (Note 5). The product is isolated from hydrochloride by treatment with excess sodium bicarbonate solution, continuous ether extraction for 3 hours and evaporation of the solvent.

(c) *Isonicotinic-C^{14} Acid Hydrazide.* A mixture of the crude ethyl isonicotinate-C^{14}, 0.23 g. of 85% hydrazine hydrate solution and 10 ml. of absolute alcohol is refluxed for 30 hours, then decolorized with carbon and evaporated to dryness. The yield of crude product is 0.328 g., 93.4% based on isonicotinic acid (Note 6). Purification is accomplished by vacuum sublimation (130° at 10 μ) and recrystallization from butyl alcohol at 0°; yield 0.260 g., m.p. 166–168°.

B. Notes

1. The method and apparatus are those described for the preparation of nicotinic-C^{14} acid; also see *o*- and *p*-aminobenzoic-C^{14} acids. A review of the halogen-metal interconversion reaction is presented by Jones and Gilman.[1]

2. The very unstable 4-bromopyridine is prepared in 85–95% yield by an adaptation of the Craig[2] modification of the Sandmeyer procedure for 2-aminopyridines. To an ice-cold, stirred solution of 4.00 g. (42.5 mmoles) of 4-aminopyridine[3,4] and 24 ml. of redistilled 48% hydrobromic acid, in a 100-ml. flask, is added 20.4 g. (128 mmoles) of bromine over a period of 10 minutes. The slurry of perbromide is diazotized at −10°, by addition of 7.57 g. (107 mmoles) of sodium nitrite in 11 ml. of water over a period of 30 minutes, then stirring for 10 minutes. The vigorously stirred mixture is warmed slowly to room temperature while copious brown fumes are evolved. The stirred mixture is cooled in an ice-bath, decolorized by the addition of a saturated sodium sulfite solution, then transferred with an equal volume of water to a 500-ml. flask, and the sulfur dioxide is expelled by heating for 7–10 minutes under reflux. The cooled solution is strongly basified with saturated sodium hydroxide, and the mixture is steam-distilled, without delay. The distillate is col-

lected in a small separatory funnel, and the colorless oil (4.5 ml., 6.539 g., 97.3% yield) is separated quickly and diluted with 100 ml. of absolute ether. The product rapidly decomposes to a yellow, ether-insoluble, water-soluble compound, unless it is dispersed in a solvent. The solution is made anhydrous by drying over magnesium sulfate, then by stirring below room temperature with 30 g. of pulverized Drierite for a minimum fo 24–36 hours. The solution is filtered by gravity through a large sintered glass funnel, protected by drying tubes, and is stored in a refrigerator. The solution is assayed by adding a 2-ml. aliquot to 20 ml. of absolute ether saturated with mercuric chloride, then collecting and washing the precipitate ($C_5H_4NBr \cdot HgCl_2$, m.p. 273° dec.) with 2–3 ml. of ether; yield 95.3%.

The 4-bromopyridine is stored conveniently as the hydrochloride, m.p. 237–239° (in a sealed capillary).

3. Four trial preparations gave crude product (83.5–96.5% yield) melting with decomposition within the range 301–304°, while a purified authentic specimen decomposed at 305°.

4. The procedure is adapted from that described by Clemo and Hoggarth.[5]

5. Ethyl isonicotinate hydrochloride melts at 154° and is easily sublimed in vacuum.[5] Ethyl isonicotinate boils at 113° (30 mm.) and forms a picrate, m.p. 142°.

6. Four trial preparations gave crude product, m.p. 165–168°, in 91.6–99.0% yield based on pure isonicotinic acid; an authentic purified specimen melted at 168–169.5°. The yield from pure ethyl isonicotinate was quantitative. The chromatography and determination of isoniazid is discussed by Albert.[6]

C. Other Preparations

By the same procedure from isonicotinic-2-C^{14} acid has been prepared[7] isonicotinic-2-C^{14} acid hydrazide; m.p. 160°, yield 7.2% based on potassium cyanide-C^{14}.

[1]*Organic Reactions*, Vol. VI, Wiley, New York, 1951, Chapter 7.
[2]L. C. Craig, J. Am. Chem. Soc., *56*, 232 (1934).
[3]D. G. Leis and B. C. Curran, J. Am. Chem. Soc., *67*, 79 (1945).
[4]J. P. Wibaut, J. Overhoff and H. Geldof, Rec. trav. chim., *54*, 807 (1935).
[5]G. R. Clemo and E. Hoggarth, J. Chem. Soc., *1941*, 41.
[6]A. Albert and C. W. Rees, Biochem. J., *61*, 128 (1955).
[7]L. Pichat, C. Baret and M. Audinot, Bull. soc. chim. France, (5) *21*, 88 (1954); L. Pichat, C. Baret, M. Audinot, M. Herbert and J. Lambin, *Radioisotope Conference, 1954*, Vol. I, Academic Press, New York, 1954, p. 245.

1-FORMYL-C^{13}-2-PHENYLHYDRAZINE

$$HC^*OOH \xrightarrow{(NH_2)_2CO} HC^*ONH_2 \xrightarrow{C_6H_5NHNH_2} HC^*ONHNHC_6H_5$$

H. R. V. Arnstein and R. Bentley, J. Chem. Soc., *1951*, 3509.

A. Procedure (Note 1)

Formic-C^{13} acid is mixed with an excess of urea and heated at 140–150° for 90 minutes in a stream of purified nitrogen (Note 2). The residue is vacuum-distilled, and the formamide-C^{13} (Note 3) is heated at 135° with an equal volume of phenylhydrazine as long as ammonia is evolved (15 minutes). The mixture is stored overnight; then the crystalline product is washed with water and 2 *N* hydrochloric acid, and recrystallized from ethanol. The 1-formyl-C^{13}-2-phenylhydrazine melts at 144° and contains the same atom percent excess isotope as the starting material (Note 4).

B. Notes

1. The procedure is adapted from the method of Cherbuliez[1], and is incidental to a study of the mechanism of amide formation.

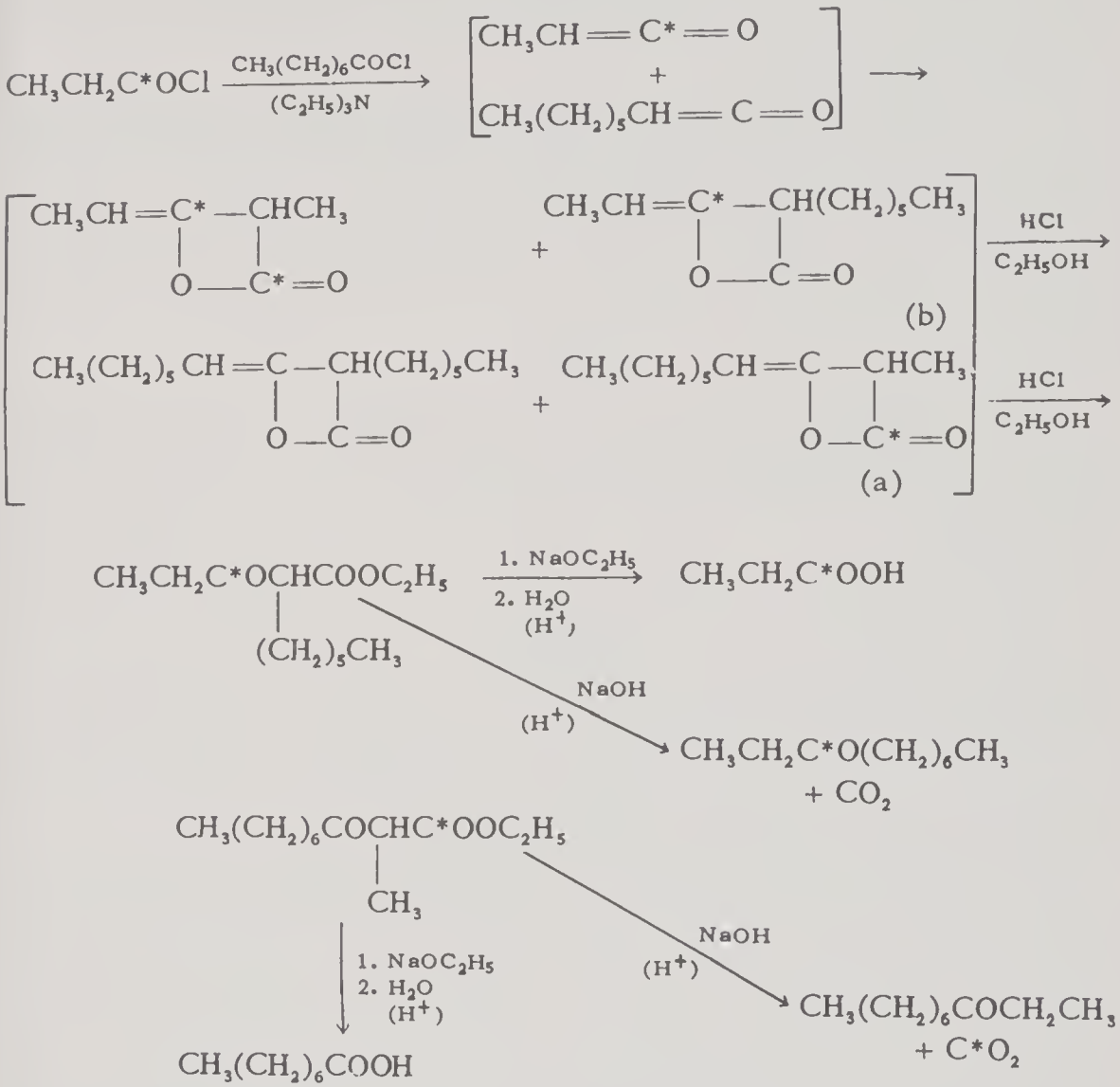
2. The evolved carbon dioxide is trapped as barium carbonate and found to contain no excess isotope.

3. The yield[1] is about 84%. The exchange between formamide and a formamido-C^{13} group has been studied by Cavalieri; see adenine-8-C^{14}.

4. The mechanism of amide formation appears to be one of simple acidolysis,[1] with no intermediate complex decomposing with the loss of carbon dioxide by alternate paths.

[1]E. Cherbuliez and F. Landolt, Helv. Chim. Acta, *29*, 1438 (1946).

HEXYLKETENE METHYLKETENE-1-C^{14} DIMER



J. D. Roberts, R. Armstrong, R. F. Trimble, Jr. and M. Burg, J. Am. Chem. Soc., *71*, 843 (1949).

A. Procedure (Note 1)

Purified triethylamine (80.9 g., 0.8 mole, Note 2) is added dropwise over a period of 3 hours to a stirred solution under nitrogen of 37 g. (0.4 mole) of propionyl-1-C^{14} chloride and 65 g. (0.4 mole) of octanoyl chloride in 500 ml. of anhydrous ether, in a 2-l. three-necked flask equipped with a Dry Ice condenser, sealed stirrer and dropping funnel. After standing for 3 days at room temperature, the amine salt is filtered off, and the ethereal solution is concentrated under reduced pressure and vacuum-distilled (0.1 mm.) into a Dry Ice-cooled receiver. The distillate is fractionated under reduced pressure with use of a Vigreux column equipped with a total condensation, partial take-off distilling head. The

yields are: methylketene-1-C^{14} dimer, 5.5 g. (24.5%), b.p. 41–49° (11.5 mm.); hexylketene dimer, 12.0 g. (24%), b.p. 148° (1.3 mm.); and mixed hexylketene methylketene-1-C^{14} dimer, 18.6 g. (25.5%), b.p. 86–89.5° (1.3 mm.) (Note 3).

Degradation studies indicate that less than 3% of the mixed dimer has a 1,3-cyclobutanedione structure, 67% of the product is in the 3-heptylidene-2-methyl-β-propiolactone-1-C^{14} (a), or its equivalent β-crotonolactone structure, and 33% in the 3-ethylidene-2-hexyl-β-propiolactone-3-C^{14} form (b), or its equivalent β-crotonolactone structure (Note 4).

B. Notes

1. The preparation is incidental to a study of the structure of a mixed aldoketene dimer prepared by the Wedekind reaction.[1,2] The structure is not the alkyl-1,3-cyclobutanedione configuration assigned to the ketoketene dimers.[2]

2. The synthesis of aldoketene dimers is by the dehydrohalogenation of acid halides as described by Sauer.[3,2] The intermediate ketene monomers rapidly undergo self-addition, or dimerization, probably under the catalytic influence of tertiary amine and tertiary amine hydrochloride.

3. Intermediate fractions total 7.1 g., and 5.2 g. of residue remains. The yield of pure dimer from unmixed acid chlorides, by this method, is reported[3] to be: methyl- 74%, and hexyl- 56%.

4. Reaction of the mixed dimer with alcohol produces two substituted acetoacetic esters, ethyl 2-propionyloctanoate and ethyl 2-octanoylpropionate, which are converted by acid cleavage to nonisotopic octanoic acid and propionic-1-C^{14} acid. The 1,3-cyclobutanedione structure, because of two chemically equivalent carbonyl groups, however, would be expected to form isotopic octanoic acid under these conditions. The lactone structures for these aldoketene dimers is in accord with other physical properties. Degradation of an aliquot of the mixed β-keto esters by ketone cleavage liberates carbon-C^{14} dioxide (equivalent to 67% of the specific activity) which must arise from structure (a). Further evidence for the structure of alkylketene dimers has been provided by the reduction studies of Wear[4] and Hill,[5] in which glycols were formed, and by the spectral and appearance potential data of Long.[6]

[1]E. Wedikind, Ber., *34*, 2070 (1901); Ann., *323*, 246 (1902); *378*, 261 (1910); E. Wedikind and J. Haeussermann, Ber., *41*, 2297 (1908).

[2]*Organic Reactions*, Vol. III, Wiley, New York, 1946, Chapter 3, p. 127; E. B. Reid and S. J. Groszos, J. Am. Chem. Soc., *75*, 1655 (1953).

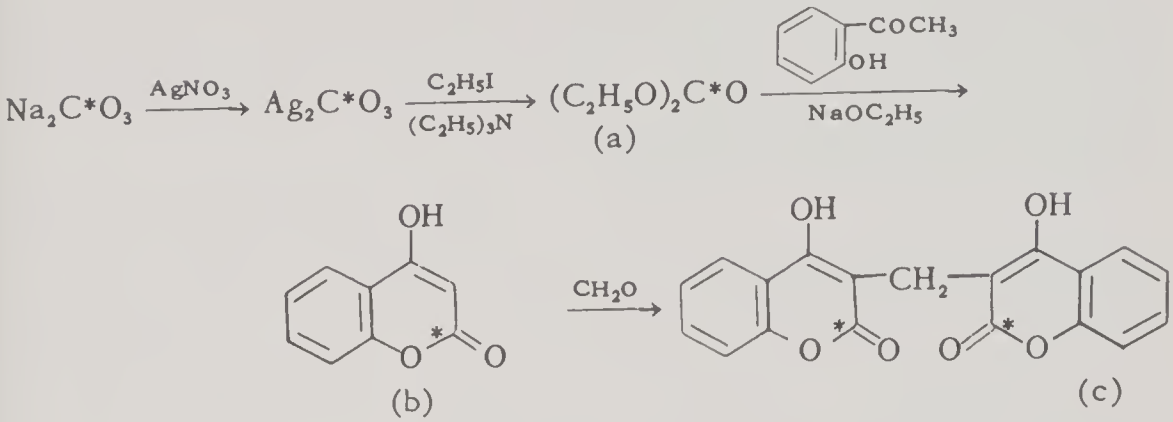
[3]J. C. Sauer, J. Am. Chem. Soc., *69*, 2444 (1947); U.S. 2,238,826, Chem. Abstracts, *35*, 4970 (1941).

[4]R. L. Wear, J. Am. Chem. Soc., *73*, 2390 (1951).

[5]C. M. Hill, L. Haynes and M. E. Hill, *ibid.*, *74*, 3423 (1952).

[6]F. A. Long and L. Friedman, J. Am. Chem. Soc., *75*, 2837 (1953).

3,3′-METHYLENEBIS(4-HYDROXYCOUMARIN-2-C^{14}) (C_2^{14}-Dicumarol)



H. R. Eisenhauer, J. M. Pepper, L. B. Jaques and J. W. T. Spinks, Can. J. Chem., *30*, 245 (1952).

A. Procedure

(a) *Ethyl Carbonate-C^{14}.* A mixture of the silver carbonate-C^{14} prepared from 0.104 g. of barium carbonate-C^{14} (Note 1), 3.0 ml. of ethyl iodide and 0.1 ml. of triethylamine (Note 2) is refluxed for 2 hours at 100° in a 4-ml. flask, while dry nitrogen is bubbled in and exhausted through a sodium hydroxide trap. The mixture is diluted with 0.10 ml. of carrier ethyl carbonate and filtered, the residue being washed with five 1-ml. portions of anhydrous benzene. The solutions are combined and slowly fractionated through a small Vigreux column attached to a flask equipped with a dropping funnel and thermometer. Sufficient anhydrous benzene is added to maintain the liquid level at 2 ml., while the distillation is continued for 10 minutes after the vapor temperature reaches the boiling point of benzene.

(b) *4-Hydroxycoumarin-2-C^{14}* (Note 3). To the benzene solution of ethyl carbonate-C^{14} is added a solution of 0.023 g. of sodium in 0.5 ml. of absolute ethanol, and 0.060 ml. of 2′-hydroxyacetophenone. The alcohol is slowly distilled, as the azeotrope, while dry benzene is added to keep the liquid level constant. When the distillate reaches the boiling point of benzene, heating is continued for 2.5 hours under reflux. Water (7 ml.) is added, and the separated aqueous phase is acidified under nitrogen with 1.0 ml. of 6 *N* hydrochloric acid (Note 4).

(c) *3,3′-Methylenebis(4-hydroxycoumarin-2-C^{14}).* To the suspension of 4-hydroxycoumarin-2-C^{14}, heated to solution under reflux at 100°, is added 2 ml. of 38% formaldehyde solution. The mixture is stored overnight, and the product is collected, air-dried and recrystallized from cyclohexanone; yield 0.0122 g., m.p. 287–288°.

B. Notes

1. The carbon-C^{14} dioxide is absorbed in the stoichiometric amount of sodium hydroxide (2.0 ml., 0.5127 *N*), which is then stirred into a solution of 0.185 g. of silver nitrate and 20 ml. of water. The precipitate is filtered off, washed with water, acetone and ether, then dried for 2 hours at 20° (1 mm.) and finally powdered.

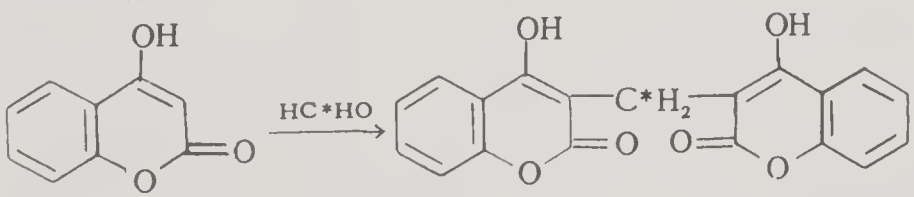
2. The yield is 9% when equivalent amounts of ethyl iodide and silver carbonate are heated in a sealed tube. The yield rises to 30% if catalytic amounts of triethylamine or pyridine are employed under conditions of reflux. A more efficient synthesis might be achieved through the reaction of phosgene-C^{14} and absolute ethanol.[1]

3. The procedure is adapted from the method of Dickenson[2] for preparing cyclic lactones of the coumarin series.

4. The precipitated 4-hydroxycoumarin may be filtered off in 68% yield based on 2′-hydroxyacetophenone. No product is formed unless ethyl carbonate is present in excess. The preferred molar ratio of reactants is 1.0 ethyl carbonate, 1.25 sodium ethylate and 0.62 2′-hydroxyacetophenone.

[1]H. Lux, Ber., *62B*, 1824 (1929).
[2]H. G. Dickenson, U. S. 2,449,162; Chem. Abstracts, *43*, 694 (1949).

3,3′-METHYLENE-C^{14}-BIS(4-HYDROXYCOUMARIN) (C_1^{14}-Dicumarol)



A. Murray III, C. W. Bills and A. R. Ronzio, J. Am. Chem. Soc., *74*, 2405 (1952).

A. Procedure

To a clear refluxing solution of 3.60 g. (22.2 mmoles) of 4-hydroxycoumarin (Note 2) in 600 ml. of water is added 9.00 mmoles of formaldehyde-C^{14} in 60 ml. of water. The mixture becomes turbid at once, and the product begins to separate within 2 minutes. The mixture is heated for 30 minutes, then filtered hot through a medium sintered glass funnel, the precipitate being washed with 500 ml. of boiling water. The crude product is dried in a vacuum desiccator, then dissolved in 50 ml. of boiling cyclohexanone, and the filtered solution is cooled slowly to crystallize the product; yield 2.944 g. (97.4%), m.p. 281–283° (dec.) (Note 3).

B. Notes

1. The procedure is an adaptation of that described by Stahmann;[1] also see Sullivan.[2] 3,3′-Methylene-C^{14}-bis(4-hydroxycoumarin) has been prepared by Lee[3,4] (70–75% yield), essentially by the procedure described. Lee[4] has determined the isotope effect (7%) involving formation of the C^{12}-C^{14} bond, and discussed the mechanism. 3,3′-Methylene-C^{14}-bis(4-coumarinyl) diacetate, m.p. 250–252° (dec.), is prepared by treating the dicumarol with acetic anhydride.

2. The reagent is prepared from methyl acetylsalicylate (22% yield) according to Stahmann.[5] The solubility in water is about 15 mg./l. at 26° and 6 mg./ml. at 92°.

3. See formaldehyde-C^{14}, Note 17.

[1]M. A. Stahmann, C. F. Huebner and K. P. Link, J. Biol. Chem., *138*, 513 (1941).

[2]W. R. Sullivan, C. F. Huebner, M. A. Stahmann and K. P. Link, J. Am. Chem. Soc., *65*, 2288 (1943).

[3]C. C. Lee, L. W. Trevoy, L. B. Jaques and J. W. T. Spinks, Can. J. Research, *28B*, 170 (1950).

[4]C. C. Lee and J. W. T. Spinks, Can. J. Chem., *32*, 327 (1954).

[5]M. A. Stahmann, I. Wolff and K. P. Link, J. Am. Chem. Soc., *65*, 2285 (1943).

ACETONITRILE-2-C^{14}

$$C^*H_3OH \xrightarrow{SO_3} C^*H_3OSO_2OH \xrightarrow{KCN} C^*H_3CN$$

D. N. Hess, J. Am. Chem. Soc., 73, 4038 (1951).

A. Procedure

Acetonitrile-2-C^{14}. Into an evacuated flask (Note 1), containing 10.5 mmoles of crystalline sulfur trioxide and a magnetic stirring bar, is distilled 10.06 mmoles of methanol-C^{14} by cooling with liquid nitrogen. The mixture is stirred with ice-bath cooling until the initial reaction is over (Note 2), then for 30 minutes at room temperature. The flask is removed from the vacuum line, and 10 ml. of 7.5 *M* potassium cyanide is added dropwise with liquid nitrogen cooling. The mixture is slowly warmed to room temperature and stirred for 30 minutes. The product is distilled into a 40-ml. flask with successive addition of three 10-ml. portions of water to ensure quantitative transfer. The radiochemical yield is 96% (Note 3).

B. Notes

1. The flask consists of a 25-ml. upper bulb, with attachment to a vacuum manifold, and a 10-ml. lower bulb.

2. Completion of reaction is indicated by the absence of methanol vapor pressure.

3. The product is assayed by hydrolysis to acetic-2-C^{14} acid in 91% yield from methanol. The acid and a typical derivative were analyzed for purity by dilution technique[1] and found to be pure within the limits of the method, estimated to have an error of ± 1%.

A preparation by this method has been described by Kögl.[2]

C. Other Preparations

Acetonitrile-2-C^{14} has been prepared in 70% yield by Brown,[3] by reacting 55 mmoles of methyl-C^{14} iodide with 61 mmoles of aqueous sodium cyanide in a sealed tube for 24 hours at room temperature. The product was extracted with benzene from a steam distillate collected at 76–100°.

Acetonitrile-1-C^{14} has been prepared[3] by a somewhat similar procedure[4] (although Kilmer[5] had previously reported the method unsatisfactory with sodium cyanide-C^{13}). To a solution of 4.9 g. (0.1 mole) of sodium cyanide-C^{14} in 10 ml. of water is added 12.6 g. (0.1 mole) of freshyl distilled methyl sulfate in small portions with shaking and cooling of the exothermic reaction in an ice-bath, as necessary. The solution is distilled slowly in a microdistillation apparatus, and the fraction boiling at 76–100° (8.6 ml.) is collected. The crude product is extracted into benzene and dried over sodium sulfate; yield 90%.

Acetonitrile-1-C^{13} has been prepared[6,7] from 20 mmoles of sodium cyanide-C^{13} by the action of methyl sulfate. The nitrile was hydrolyzed to sodium acetate-1-C^{13}; yield 83% based on cyanide. The preparation has also been reported by Elwyn.[7]

Acetonitrile-2-C^{14} and -2-C^{13} have been prepared[8] by refluxing for 6 hours a mixture of 1.05 ml. of methyl-C* iodide and 1.01 g. of sodium cyanide in 1.8 ml. of water. The nitrile was steam-distilled and converted to acetic-2-C^{14} acid by alkaline hydrolysis; yield 66% based on methanol. Acetonitrile-1-C^{13} has been prepared[5] by this method; see ethylamine-1-C^{13} (70% yield).

Also see sodium acetate-2-C^{14}, Method III.

[1]G. A. Ropp, J. Am. Chem. Soc., *72*, 4459 (1950).

[2]F. Kögl, P. Emmelot and D. H. W. den Boer, Ann., *589*, 1 (1954); F. Kögl and A. M. Municio, Hoppe Seyler's Z. physiol. Chem., *300*, 6 (1955).

[3]E. V. Brown, E. Cerwonka and R. C. Anderson, J. Am. Chem. Soc., *73*, 3735 (1951).

[4]P. Walden, Ber., *40*, 3214 (1907).

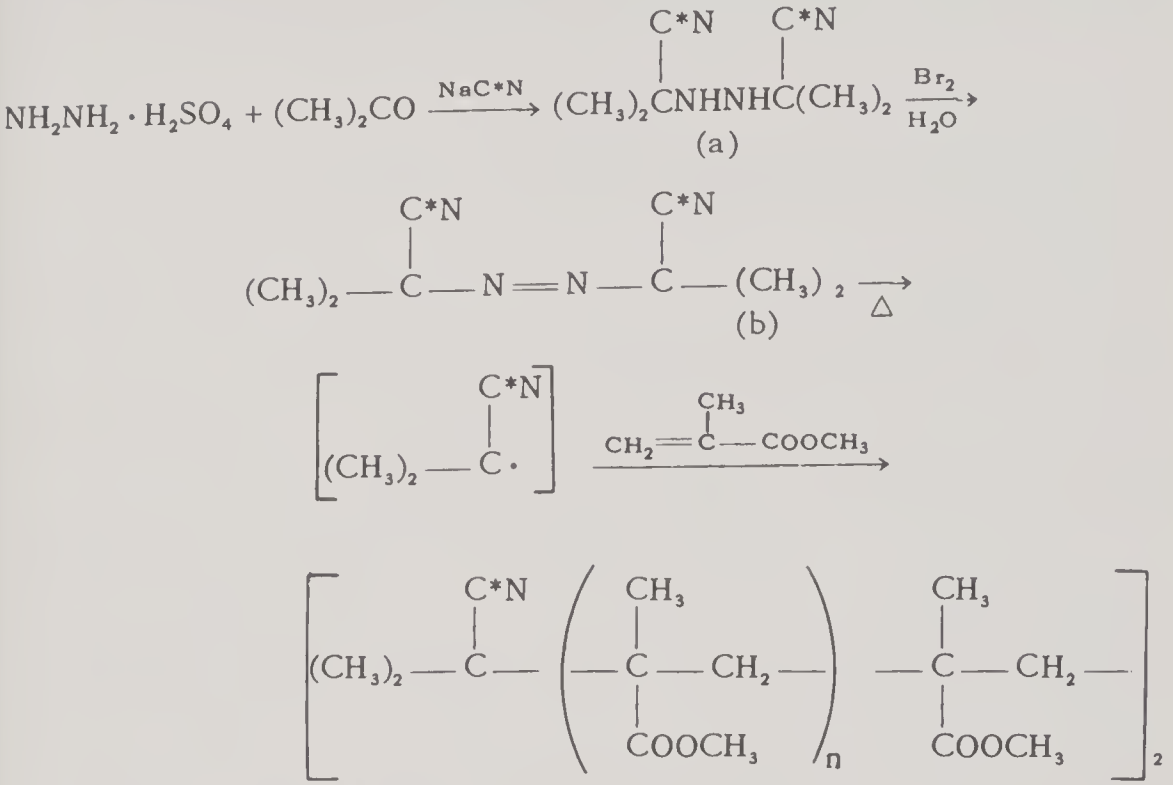
[5]G. W. Kilmer and V. du Vigneaud, J. Biol. Chem., *154*, 247 (1944).

[6]S. Weinhouse, G. Medes and N. F. Floyd, J. Biol. Chem., *158*, 411 (1945).

[7]D. Elwyn and D. B. Sprinson, J. Biol. Chem., *184*, 465 (1950).

[8]H. N. Little and K. Bloch, J. Biol. Chem., *183*, 33 (1950).

2,2′-AZOBIS(2-METHYLPROPIONITRILE-1-C^{14})



L. M. Arnett and J. H. Peterson, J. Am. Chem. Soc., 74, 2031 (1952).

A. Procedure

(a) *2,2′-Hydrazobis(2-methylpropionitrile-1-C^{14})*. The compound is synthesized from sodium cyanide-C^{14} by the method of Thiele and Heuser,[1] which follows.

A solution of 130 g. of hydrazine sulfate in a minimum amount of warm water is mixed vigorously with a concentrated aqueous solution of 130 g. of potassium cyanide (2 moles) and 116 g. of acetone (2 moles) in a flask fitted with a reflux condenser (Note 1). After a time the mixture becomes turbid, and an oily layer forms. Gradually, with cooling, a good yield of glistening white platelets precipitates (Note 2). After 12 hours, the solid is filtered off and washed with water. The product is quite pure, m.p. 92–93°, after recrystallization from ether (Note 3).

(b) *2,2′-Azobis(2-methylpropionitrile-1-C^{14})*. The compound is synthesized by oxidation of 2,2′-hydrazobis(2-methylpropionitrile-1-C^{14}) by the method of Thiele and Heuser,[1] which follows.

The crude 2,2′-hydrazobis(2-methylpropionitrile) is dissolved, without heating, in a minimal volume of alcohol and diluted with two volumes of dilute hydrochloric acid; the mixture is cooled with ice and vigorously stirred while bromine water is added dropwise until a yellow color persists; about 1660 ml. of bromine water (50 g. bromine) is consumed. The insoluble product is collected, washed and crystallized from ether,

or from alcohol by dilution with water; yield 50 g. (Note 4). The purified colorless compound melts at 105–106° with regular quantitative nitrogen evolution and the formation of tetramethylsuccinonitrile.

B. Notes

1. Gradual mixing with initial cooling is advisable.

2. Overberger and co-workers[2] shook the mixture for 2 days at room temperature.

3. The compound, which may be crystallized from 95% alcohol, is sparingly soluble in ligroin, insoluble in water and alkali, soluble in dilute acid, and decomposes upon heating with water.

4. The use of isotopes in the study of vinyl polymerization is discussed by Bevington.[3] The addition polymerization of vinyl monomers is believed to proceed by a free radical mechanism and may be initated by free radicals. In peroxide-catalyzed (free radical) polymerization, which is interrupted before completion, no polymers of intermediate molecular weight are found. When the catalyst is boron fluoride products of all sizes may be isolated. There is evidence that the thermal decomposition of azobis(aliphatic-nitriles) produces such radicals,[2,4] that upward of one-half of these radicals initiate polymer chains, and that the termination reaction[5] is the combination of two such growing chains. The comparison of the osmotic molecular weight and the amount of combined labeled initiator fragments, in the case of methyl methacrylate polymer,[6] indicates that there is a label at both ends of the chain.

C. Other Preparations

By a similar procedure from 2-propanone-1,3-C_2^{14} are prepared:[6] 2,2′-hydrazobis(2-methyl-C^{14}-propionitrile-3-C^{14}) and 2,2′-azobis(2-methyl-C^{14}-propionitrile-3-C^{14}). The final product is recrystallized from alcohol, the solution being heated only to 50° for a minimum length of time to avoid decomposition.

Methyl C^{14}-Methacrylate Polymer. To 0.2096 g. of 2,2′-azobis(2-methylpropionitrile-1-C^{14}), contained in a 19-mm. Pyrex glass tube with a constriction 12 cm. from the bottom, is added 15.45 g. of alkali-washed, redistilled (46° at 100 mm.) methyl methacrylate by vacuum distillation. The tube is sealed off under vacuum, shaken to assure homogeneity and heated at 50° for 55 minutes, then chilled in an ice-bath (Note 6). The tube contents are washed out with benzene into 500–600 ml. of methanol stirred in a Waring Blendor. The flocculent precipitate is collected by filtration, washed with methanol and dried at room temperature for at least 4 hours, and to constant weight at 60–65°. The product is purified by dissolution in benzene and reprecipitation (3–4 times) to constant specific activity. The yield under these conditions is 1.683 g. (molecular

weight 390,000) containing 0.707 mg. of labeled initiator, 52% of that which has decomposed under these conditions. The loss of low molecular weight fractions is calculated to be of the order of 0.1%. Kinetic studies[5] show that the initiation reaction is virtually independent of monomer concentration, and nearly so of temperature.

A series of vinyl polymers is prepared by the same procedure with the indicated initiator efficiency, solvent and precipitant:

C^{14}-Polystyrene, 66–74%, chloroform, methanol. At 66° the efficiency becomes 81.5%.

The use of styrene-β-C^{14} in studying the synthesis of branched polymers of both high and low molecular weight has been reported by Jones[6] and Bevington.[7]

The polymerization of styrene in the presence of benzoyl-C^{14} peroxide has been studied by Koton.[8] Solid polymers (mol. wt. 19,400–30,600) having 1.3–1.73 benzoyl-C^{14} radicals per molecule were obtained.

C^{14}-Polyacrylonitrile, 103% in 75% ethanol solution, *N,N*-dimethylformamide, methanol.

C^{14}-Polyvinyl acetate, 83%, acetone, hexane. The first precipitation is in methanol-water (1 : 3).

The use of polyvinyl acetate-1-C^{14} in studying the reactions by which side-chains are introduced into polyvinyl acetate (kinetics and extent) has been reported by Bevington.[7] Polyvinyl acetate was hydrolyzed to polyvinyl alcohol, then reacetylated with a mixture of acetic anhydride and sodium acetate-1-C^{14} in the presence of pyridine; see bis(acetic-1-C^{14}) anhydride. The retardation mechanism of polymerization of vinyl acetate in aromatic solvents has been studied by Stockmayer.[9] Benzene-C_1^{14} was incorporated into the low conversion polyvinyl acetate (20 benzene molecules/average molecule).

C^{14}-Polyvinyl Chloride, 70–77% in 52–67% acetone solution, cyclohexanone, methanol.

[1]J. Thiele and K. Heuser, Ann., *290*, 1 (1896).

[2]C. G. Overberger, M. T. O'Shaughnessy and H. Shalit, J. Am. Chem. Soc., *71*, 2661 (1949).

[3]J. C. Bevington and H. W. Melville, *Radioisotope Conference, 1954*, Vol. II, Academic Press, New York, 1954, p. 3.

[4]F. M. Lewis and M. S. Matheson, J. Am. Chem. Soc., *71*, 747 (1949).

[5]L. M. Arnett, J. Am. Chem. Soc., *74*, 2027 (1952); L. M. Arnett and J. H. Peterson, J. Am. Chem. Soc., *74*, 2031 (1952).

[6]M. H. Jones, H. W. Melville and W. G. P. Robertson, Nature, *174*, 78 (1954); J. C. Bevington, G. M. Guzman and H. W. Melville, Proc. Roy. Soc. (London), *221A*, 453 (1954).

[7]J. C. Bevington, G. M. Guzman, and H. W. Melville, Proc. Roy. Soc. (London), *221A*, 437 (1954); J. C. Bevington, H. W. Melville and R. P. Taylor, J. Polymer Sci., *12*, 449 (1954).

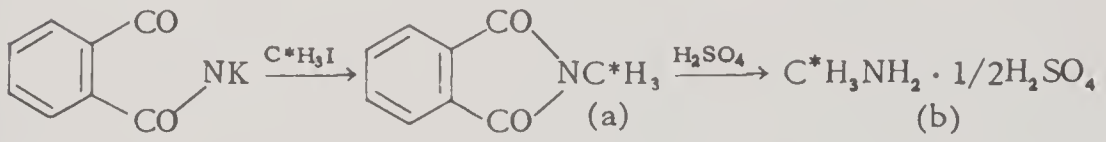
[8]M. M. Koton, T. M. Kiseleva and M. I. Bessonov, Doklady Akad. Nauk S.S.S.R., *96*, 85 (1954); Chem. Abstracts, *48*, 10374 (1954).

[9]W. H. Stockmayer and L. H. Peebles, Jr., J. Am. Chem. Soc., *75*, 2278 (1953).

AMINES

METHYLAMINE-C*

METHOD I



J. D. Cox and R. J. Warne, J. Chem. Soc., *1951*, 1896.

A. Procedure

(a) N-*Methyl-C*^{13}*-phthalimide.* Dry methyl-C^{13} iodide (2.84 g., 20 mmoles) is vacuum-distilled, with liquid nitrogen cooling, into the side tube of a flask (Note 1) containing 5.0 g. (27 mmoles) of potassium phthalimide,[1] which has been previously dried by heating at 200° for 1 hour under vacuum. The flask is sealed off at a constriction in the neck, then heated at 180° until no liquid remains in the side tube, which is maintained at room temperature (6–48 hours). The contents are triturated with a minimal amount of ice-cold water, 0.1 *N* potassium hydroxide and then water, leaving a product requiring no purification; yield 3.19 g., 98% (Note 2).

(b) *Methylamine-C*^{13} *Sulfate.* A mixture of 3.19 g. (19.6 mmoles) of *N*-Methyl-C^{13}-phthalimide and 7 ml. of constant-boiling hydrochloric acid is refluxed for 4 hours, the sublimate in the condenser being washed down occasionally with acid. The mixture is cooled in an ice-bath, basified with sodium hydroxide and distilled in a Kjeldahl apparatus; the product is collected under 19.00 ml. of 1.038 *N* sulfuric acid. The yield is 19.6 mmoles (100%), determined by titrating the excess acid with 1.00 *N* methylamine solution (0.18 ml.).

METHOD II

$$HC^*N \xrightarrow[\substack{PtO_2\\HCl}]{H_2} C^*H_3NH_2\cdot HCl$$

R. D. H. Heard, J. R. Jamieson and S. Soloman, J. Am. Chem. Soc., 73, 4985 (1951).

A. Procedure (Note 3)

Methylamine-C^{14} Hydrochloride. An aqueous solution of 0.205 g. (4.18 mmoles) of sodium cyanide-C^{14} and 2.24 molar proportions excess of sodium hydroxide is evaporated to dryness under vacuum at 30° in a 125-ml. flask. The flask then is attached, through a closed, wide-bore stopcock, to a horizontal reservoir (30 ml.) containing 5 ml. of glacial acetic acid and 0.085 g. of freshly prepared Adams catalyst[2] (Note 4). The catalyst is reduced with hydrogen which has been generated from arsenic-free zinc and reagent hydrochloric acid (Kipp generator), passed through 20% solutions of potassium hydroxide, silver nitrate and saturated potassium permanganate, then stored in a 250-ml. burette. To the reservoir is added a solution of 0.96 ml. (11.5 mmoles) of concentrated hydrochloric acid in 5 ml. of acetic acid (Note 5). The system is refilled with hydrogen, the apparatus is tilted, and the stopcock is opened to add the contents of the reservoir to the reaction flask. Hydrogenation is continued with shaking at room temperature and slight positive pressure until absorption ceases (Note 6). The catalyst is filtered off and washed with dilute hydrochloric acid. The combined filtrates are evaporated to dryness under vacuum. The residue consists of a mixture of sodium chloride and methylamine-C^{14} hydrochloride (better than 85% yield), suitable for conversion to diazomethane-C^{14} without purification (Note 7). Purification is effected best by distillation of the free amine from alkali and collection under dilute hydrochloric acid (Note 8).

METHOD III

$$C^*H_3C^*OCl \xrightarrow{NaN_3} C^*H_3C^*ON_3 \rightarrow C^*H_3NC^*O \xrightarrow{HCl} C^*H_3NH_2\cdot HCl$$

E. M. Gal, R. E. Spenger, and D. M. Greenberg, J. Org. Chem., *15*, 1261 (1950).

A. Procedure (Note 9)

Methylamine-C^{14} Hydrochloride. The apparatus consists of a pear-shaped flask equipped with a dropping funnel and a water-cooled reflux condenser surmounted by a low-temperature condenser cooled with Dry Ice-alcohol. To a mixture of 3.87 g. (49.3 mmoles) of acetyl-C_2^{14} chloride (Note 10) and 12 ml. of benzene at −78° is added 3.85 g. (59.2 mmoles)

of sodium azide (Note 11). The system is closed (upper condenser in operation), and the mixture is stored overnight at room temperature to form methyl-C^{14} isocyanate-C^{14} by rearrangement[3] of the azide. The system is fitted with a carbon dioxide trap containing 16 g. of crystalline barium hydroxide in 400 ml. of water, and protected from the atmosphere by a soda-lime tube. The mixture is cooled to 0°, treated with 12 ml. of concentrated hydrochloric acid, heated at 75° for 6 hours, then stored overnight at room temperature with the trap closed off (Note 12). The mixture is evaporated to dryness under reduced pressure at 100°. The residue is extracted with three 25-ml. portions of absolute ethanol, and the solvent is evaporated under vacuum. The residue is extracted with four 50-ml. portions of isopropyl alcohol, and the combined extracts are concentrated to small volume. Absolute ether (250 ml.) is added to precipitate the product, which is collected by centrifuging and dried in a desiccator; yield 2.58 g. (38.2 mmoles), 76%, m.p. 226–227°.

B. Notes

1. A diagram of the flask, in the form of a "Faraday tube," is presented by Cox and Warne. The methyl iodide is dried by milling with phosphorus pentoxide and glass beads in a closed tube.

2. Titration with silver nitrate shows that the aqueous extract contains 19.6 mmoles of iodide ion. The product is readily transferred by dissolution in chloroform. The yield falls to 70%, but the titrated iodide ion remains at 99%, if the methyl iodide is not dried as described.

3. Jones and Skraba[4] have reported the hydrogenation of potassium cyanide-C^{14} at atmospheric pressure in dilute sulfuric acid solution over platinum oxide. Methylamine-C^{14} hydrochloride was obtained in 98% yield by making the reaction mixture basic, and distilling the amine into a receiver containing hydrochloric acid. The product gave diazomethane-C^{14} in 54% yield based on cyanide.

4. A diagram of the apparatus is given by Heard. Aged platinum oxide and certain tank hydrogens, even scrubbed as described, are unsuitable.

5. Hydrochloric acid arrests the reduction at the mono-amine stage.

6. The theoretical 2 moles of hydrogen is absorbed in 4–7 hours. The precipitated sodium chloride is not detrimental to the preparation of diazomethane.

7. The crude salt yields diazomethane-C^{14} in 41–42% yield based on sodium cyanide.

8. Extraction of the crude salt with hot butanol, followed by concentration to 3 ml. and crystallization in a refrigerator, gives a recovery of only 33%, m.p. 222–224°.

9. The procedure is adapted from the procedure of Naegeli.[5] A diagram of the apparatus is given by Gal.

10. Despite the fact that carbon-C^{14} dioxide is liberated from C-1 by the Curtius reaction, the doubly labeled acid was used by these investigators because of its availability to them. The normal procedure would be to employ acetic-2-C^{14} acid.

11. Sodium azide prepared from hydrazine and ethyl nitrite, according to Thiele,[6] appears to give better results[5] than the commercial product. The latter may be activated by the method of Nelles.[7]

12. The yield of recovered barium carbonate-C^{14} is 6.63 g. (33.7 mmoles).

C. Other Preparations

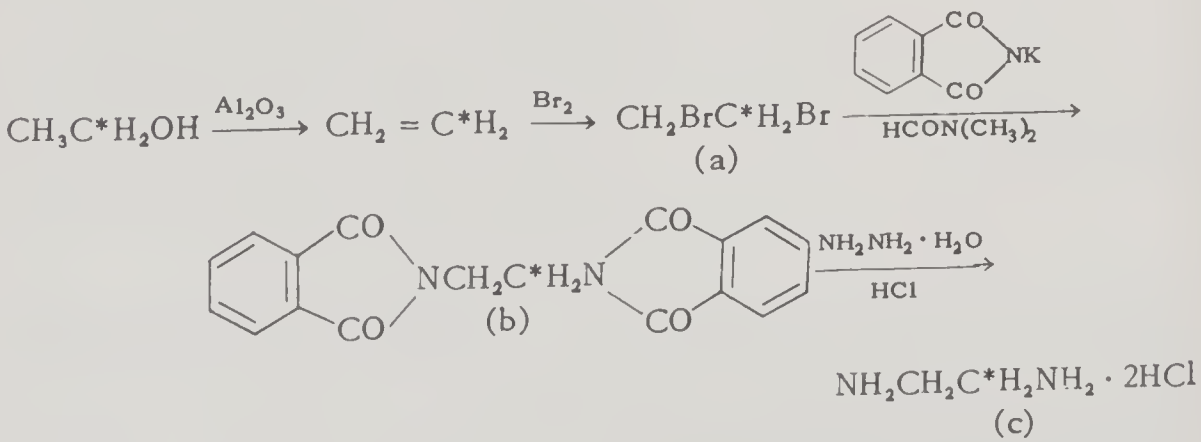
Methylamine-C^{13} hydrochloride has been prepared by Hershberg,[8] by reducing sodium cyanide-C^{13} (2.3 g.) with excess chromous chloride solution at pH 7–7.5 at 80–85° for 1 hour in a hydrogen atmosphere. The yield of purified product, crystallized from 100 ml. of butanol, was 1.09 g., 37% (see Method II, Notes 4 and 5.) The crude product, however gave diazomethane-C^{13} in 30% yield based on sodium cyanide.

The preparation of methylamine-C^{14} hydrochloride by the method of Jones and Skraba (Note 3) has been reported by Schayer.[9]

[1] *Organic Syntheses,* Coll. Vol. I, Wiley, New York, 1941, p. 119.
[2] *Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 463.
[3] *Organic Reactions*, Vol. III, Wiley, New York, 1946, p. 337.
[4] A. R. Jones and W. J. Skraba, Science, *117*, 252 (1953).
[5] C. Naegeli, L. Gruntuch and P. Lendorff, Helv. Chim. Acta, *12*, 227 (1929).
[6] J. Thiele, Ber., *41*, 2681 (1908).
[7] J. Nelles, Ber., *41*, 1345 (1932).
[8] E. B. Hershberg, E. Schwenk and E. Stahl, Arch. Biochem., *19*, 300 (1948).
[9] R. W. Schayer, R. L. Smiley and E. H. Kaplan, J. Biol. Chem., *198*, 545 (1952).

C^{14}-ETHYLENEDIAMINE

METHOD I



D. S. Popplewell and R.G. Wilkings, J. Chem. Soc., *1955*, 2521.

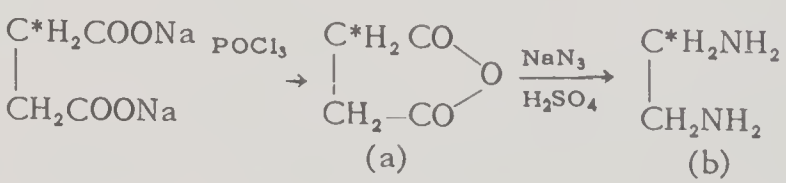
A. Procedure

(a) *1,2-Dibromoethane-C_1^{14}*, (*Ethylene-C_1^{14} Bromide*) (Note 1). Ethanol-1-C^{14} (2.8623 g.) is distilled during 2.5 hours through a 17-in. tube containing activated alumina (100–200 mesh) heated at 360–400°. The ethylene-C_1^{14} formed (75–80%) is absorbed in a spiral trap containing 12 g. of bromine, the system being swept with a slow stream of nitrogen for 5 minutes at the end of the reaction. The product is decolorized by washing with 2 *N* sodium hydroxide and dried; yield 8.8 g. (Note 2).

(b) N,N′-(*Ethylene-C_1^{14}*)*diphthalimide*, (*1,2-Diphthalimidoethane-C_1^{14}*) (Note 3). A mixture of 8.3 g. of 1,2-dibromoethane-C_1^{14}, 20 g. of recrystallized potassium phthalimide and 90 ml. of dimethylformamide is heated on a steam-bath for 4 hours. The mixture is concentrated and poured onto ice, and the product is collected and washed with water; yield 7.45 g., m.p. 228–232°. Concentrating the filtrate gives a second crop, 4.3 g. (m.p. 195–215°). The combined solid is purified by boiling with carbon disulfide to extract any *N*-(2-bromoethyl-$C_{1/2}^{14}$)phthalimide; yield 11.7 g. (Note 4).

(c) *Ethylenediamine-C_1^{14} Dihydrochloride* (Note 5). A mixture of 11.7 g. of the diphthalimide, 300 ml. of absolute ethanol and 3.3 g. of hydrazine hydrate (slight excess) is refluxed for 2–3 hours after the appearance of a precipitate (1 hour). The solvent is evaporated and the residue is heated at 100° under reduced pressure for 15 minutes, then refluxed with 100 ml. of 6 *N* hydrochloric acid for 3 hours. The mixture is evaporated under reduced pressure, and the residue is extracted with small portions of cold water. The filtrate is evaporated to dryness, and the crude product (4.9 g.) is washed with a little hot absolute ethanol and is recrystallized from concentrated hydrochloric acid; yield 4.3 g., 53.4% based on ethanol (Note 6). The over-all radiochemical yield is 52%.

METHOD II



(a) (b)

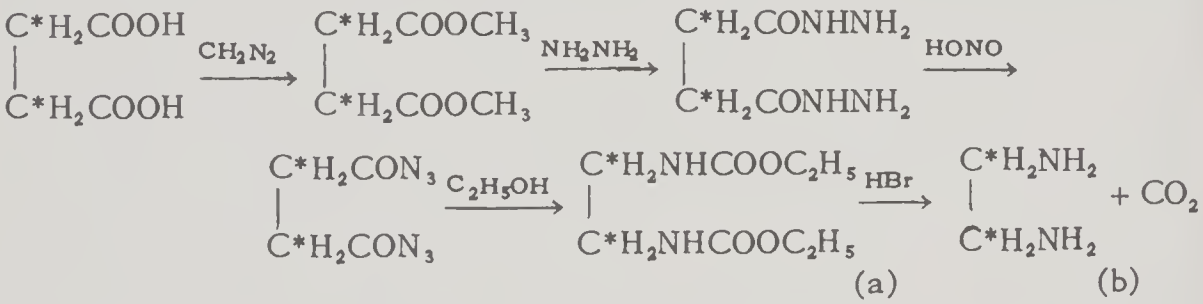
E. F. Phares and M. V. Long, J. Am. Chem. Soc., 77, 2556 (1955).

A. Procedure (Note 7)

(a) *Succinic-2-C^{14} Anhydride*. A mixture of 0.25 mmole of dry sodium succinate-2-C^{14} (Note 8) and 0.17 mmole (15 μl.) of phosphorus oxychloride is heated in a 15-ml. pear-shaped flask, fitted with a drying tube, by immersing the tip in a bath at 200° for 10 minutes. The tip is then cooled with an ice-bath, and the walls of the flask are gently flamed.

(b) *Ethylenediamine-C_1^{14} Dihydrochloride.* The mixture of crude anhydride, chlorides and phosphates is diluted with 0.25 ml. of 38 *N* fuming sulfuric acid, cooled, and treated with 0.050 g. of sodium azide with shaking. The flask is attached quickly to a train containing an acid permanganate scrubber and an alkali trap (Note 9). The tip of the flask is warmed slowly (Note 10) to initiate the vigorous reaction. When gas evolution subsides, the flask is immersed in a water-bath (70°) which is brought to boiling during several minutes; then the system is swept with carbon dioxide-free air for 5–10 minutes (Note 11). The cooled mixture is diluted with 1 ml. of water and made alkaline to phenol red by the dropwise addition of saturated sodium hydroxide. The alkaline solution is frozen as a thin film on the walls of the flask with liquid nitrogen, then is vacuum-distilled into a trap cooled with liquid nitrogen. The distillation is completed by warming with warm water while pumping is continued for 15 minutes. The distillate is titrated with hydrochloric acid (chlorphenol red) and evaporated; yield 73% based on succinic acid (Note 12).

METHOD III



A. A. Benson and J. A. Bassham, J. Am. Chem. Soc., *70*, 3939 (1948).

A. Procedure

(a) *Ethyl Ethylene-C_2^{14}-dicarbamate.* Succinic-2,3-C_2^{14} acid (0.280 g.) is converted quantitatively with diazomethane to methyl succinate-2,3-C_2^{14}. The dihydrazide is prepared from the ester in 90% yield, then converted to the diazide by the methods of Curtius[1] and Shofer[2] (Note 13). By this method the diazide is rearranged in ethanol to the product, which is recrystallized from water and vacuum-sublimed; yield 0.117 g., 36% (Note 14).

(b) *Ethylenediamine-C_2^{14} Dihydrochloride.* Ethyl ethylene-C_2^{14}-dicarbamate (0.104 g.) is refluxed for 2 hours with 5 ml. of 48% hydrobromic acid (Note 15). The mixture is evaporated under vacuum, then treated with excess methanolic potassium hydroxide. After evaporation of solvent, the ethylenediamine-C_2^{14} is vacuum-distilled and converted to the dihydrochloride by addition of methanolic hydrogen chloride; yield 0.052 g. (76%). The product may be recrystallized from methanol-water.

B. Notes

1. Dehydration of ethanol with hot syrupy phosphoric acid (see propene-1-C^{14}) gives poor yields.

2. Ethylene bromide is soluble in 250 parts of water, b.p. 131–132°, d_{25}^{25} 2.172.

3. The procedure is an adaptation of the method of Sheehan[3] for condensing potassium phthalimide with organic halides. The use of solvant raises the yields from 60% to 80–85%.

4. A sample of product recrystallized from absolute alcohol melts at 233°.

5. The method is that of Ing and Manske[4] for cleaving alkylphthalimides.

6. The picrate melts at 233°. An aqueous solution of ethylenediamine-C_1^{14} is obtained[5] by passing an aqueous solution of the dihydrochloride through a column of Amberlite IRA-400 in the hydroxy form. Ethylenediamine-C_1^{14} dinitrate is prepared in almost quantitative yield by dissolution of the dihydrochloride in a minimal volume of hot saturated lithium nitrate solution, addition of alcohol, and cooling. The product, well washed with alcohol, is chloride- and lithium-free.

7. This preparation is essentially a degradation of C^{14}-succinic acid, or the more reactive anhydride. The procedure is a modification of the Schmidt reaction, as applied to fatty acid degradation by Phares[6] and Strassman,[7] designed to increase the amine recovery above the 10% obtained by steam distillation.

8. The salt or acid is dried for 30 minutes at 105°.

9. A diagram of the apparatus is given by Phares;[6] 5% $KMnO_4$ in 1 *N* H_2SO_4, 0.5 *N* NaOH.

10. The increased reactivity of the anhydride over that of the acid makes extra care in the first warming advisable. With greater quantities cooling may be required.

11. The trapped carbon dioxide is precipitated with barium chloride for assay; yield 84% based on succinic acid.

12. The yield drops to 45% when the anhydride step is skipped. There is no melting point depression when the dibenzenesulfonamide (m.p. 168°) and dibenzamide (m.p. 224°) are mixed with authentic derivatives, indicating that no other volatile amine is formed. Ethylenediamine dihydrochloride sublimes without melting. Ethylenediamine monohydrate boils at 118°.

13. Succinyl azide is obtained readily in high yield from the acid chloride and sodium azide,[8] and the method avoids the complicating possible formation of small amounts of a cyclic secondary hydrazide.

14. No melting point is reported in this paper. Ethylene isocyanate, which is formed by rearrangement of succinyl azide, gives an imidazolidone with ethanol instead of a normal urethan.[8] Both the isocyanate and the imidazolidone, however, are hydrolyzed readily to ethylenediamine.[9]

15. As this procedure was designed as a degradative method to separate C-1 and C-2 of succinic acid, a stream of nitrogen may be passed through the solution into an alkaline bubbler to collect carbon dioxide.

C. Other Preparations

Succinic-2,3-C_2^{14} anhydride has been prepared[10] (yield 75%) by refluxing the acid with acetyl chloride.

[1]T. Curtius, J. prakt. Chem., *52*, 222 (1895).
[2]Shofer and Schwann, J. prakt. Chem., *51*, 190 (1895).
[3]J. C. Sheehan and W. A. Bolhofer, J. Am. Chem. Soc., 72, 2786 (1950).
H. R. Ing and R. H. F. Manske, J. Chem. Soc., *1926*, 2348; *Organic Syntheses*, Vol. 29, Wiley, New York, 1949, p. 18.
[5]D. S. Popplewell and R. G. Wilkins, J. Chem. Soc., *1955*, 4098.
[6]E. F. Phares, Arch. Biochem. Biophys., 33, 173 (1951).
[7]M. Strassman and S. Weinhouse, J. Am. Chem. Soc., 74, 3457 (1952); 75, 1680 (1953).
[8]C. Naegeli and P. Lendorff, Helv. Chim. Acta, *15*, 49 (1932).
[9]*Organic Reactions,* Vol. III, Wiley, New York, 1946, p. 344.
[10]R. F. Nystrom, Y. H. Loo, K. M. Mann and J. R. Allen, Nucleonics, 7 (3), 61, 57 (1950).

DIETHYLAMINE-1,1′-C_2^{14} HYDROCHLORIDE

$$CH_3C^*N \xrightarrow[Ni]{H_2} (CH_3C^*H_2)_2NH + CH_3C^*H_2NH_2 + NH_3$$

$$CH_3C^*H_2NH_2 \xrightarrow[NH_3\ Ni]{CH_3CHO + H_2} \begin{matrix} CH_3C^*H_2 \\ \diagdown \\ NH \\ \diagup \\ CH_3CH_2 \end{matrix} + CH_3CH_2NH_2$$

A. Stoll, J. Rutschmann and A. Hofmann, Helv. Chim. Acta, 37, 820 (1954).

A. Procedure (Note 1)

Into a hydrogenation flask containing 5 ml. of 50% ethanol and the Raney nickel catalyst prepared from 0.100 g. of Raney alloy (Note 2) is distilled 0.102 g. of acetonitrile-1-C^{14} under high vacuum, with liquid nitrogen cooling. The nitrile is hydrogenated at room temperature and atmospheric pressure, the calculated amount of hydrogen (115 ml.) being absorbed within 4.5 hours. The mixture is distilled into a trap containing an excess of 2 *N* hydrochloric acid. Evaporation gives 0.195 g. of

crude amine hydrochloride, which is transferred into the first tube of a Craig countercurrent distribution apparatus with 5 ml. of water. After addition of 10 ml. of ether, the free base is liberated by the addition of 5 ml. of *N* sodium hydroxide. At the end of 40 distributions between water and ether (10 ml. each), the faster moving diethylamine-1,1′-C_2^{14} is trapped in tubes 10 and 11 which contain 10 ml. of *N* hydrochloric acid each. The yield of product obtained by evaporation is 0.047 g., 35%.

The contents of tubes 1–7 are diluted with an excess of hydrochloric acid and evaporated. The residue is treated with an excess of sodium hydroxide solution, and the liberated ethylamine-1-C^{14} is distilled into the hydrogenation flask containing water and fresh Raney nickel catalyst, by cooling with a Dry Ice-acetone bath. The mixture is treated with 0.090 g. of acetaldehyde and hydrogenated until absorption ceases (1.5 hours, 35 ml.), then worked up as described above; yield 0.105 g. of diethylamine-1-C^{14} hydrochloride. The total yield of C^{14}-diethylamine hydrochloride is 0.152 g., 71% on a radiochemical basis; the molar specific activity is 126.5% of that of the starting material.

B. Notes

1. The hydrogenation of nitriles is discussed by Wagner[1] and Winans.[2] The preparation of amines by reductive alkylation is surveyed by Emerson.[3] The reduction of a mixture of ethylamine and acetaldehyde over nickel has given diethylamine and triethylamine in yields of 55% and 19%, respectively.

2. The alloy[4] is heated with 2 ml. of 40% potassium hydroxide for 30 minutes on a steam-bath; then the catalyst is washed by decantation with seven portions of water. A diagram of the two-necked hydrogenation flask is given by Stoll.

C. Other Preparations

Ethylamine-1-C^{14} and -2-C^{14} hydrochloride have been prepared[5] by degradation of the appropriate propionic acids by the Schmidt reaction; see butyric-4-C^{14} acid, Note 7.

[1]R. B. Wagner and H. D. Zook, *Synthetic Organic Chemistry*, Wiley, New York, 1953, p. 658.

[2]C. F. Winans and H. Adkins, J. Am. Chem. Soc., *54*, 306 (1932).

[3]*Organic Reactions*, Vol. IV, Wiley, New York, 1948, Chap. 3.

[4]*Organic Syntheses*, Vol. 21, Wiley, New York, 1941, p. 15; Vol. 29, Wiley, New York, 1949, p. 24.

[5]E. F. Phares, Arch. Biochem. Biophys., 33, 173 (1951).

PROPYLAMINE-1-C^{14} PERCHLORATE

$$(CH_3CH_2O)_2SO_2 \xrightarrow{NaC^*N} \underset{(a)}{CH_3CH_2C^*N} \xrightarrow[(HClO_4)]{LiAlH_4}$$

$$\underset{(b)}{CH_3CH_2C^*H_2NH_2 \cdot HClO_4} \xrightarrow{HONO} CH_3CHOHC^*H_3 + \underset{(c)}{CH_3C^*H_2C^*H_2OH}$$

J. D. Roberts and M. Halmann, J. Am. Chem. Soc., 75, 5759 (1953).

A. Procedure

(a) *Propionitrile-1-C^{14}*. To a solution of 25 g. of sodium cyanide-C^{14} in 50 ml. of water and 50 ml. of ethylene glycol at 35° is added dropwise 66 ml. of ethyl sulfate. The mixture is stored overnight; then the low boiling constituents are distilled, and the distillate is diluted with 20 ml. of 18 *N* sulfuric acid. The upper layer is separated, dried over calcium chloride and distilled; yield 12.1 g. (44%), b.p. 94–97° (Note 1).

(b) *Propylamine-1-C^{14} Perchlorate* (Note 2). To a stirred solution of 9 g. of lithium aluminum hydride in 300 ml. of absolute ether at 0° is added dropwise a solution of 12.1 g. of propionitrile-1-C^{14} in 30 ml. of dry ether. After 3 hours, the mixture is treated cautiously with 8 ml. of water followed by 6 ml. of 20% sodium hydroxide solution and 25 ml. of water. The mixture is distilled, the distillate being collected under 20 ml. of 70% perchloric acid and evaporated to dryness at 30–35° under reduced pressure. The product is recrystallized from hexyl alcohol-heptane; yield 11 g. (31%), m.p. 167°.

(c) *1-Propanol-1,2-$C^{14}_{1/2}$* (Note 3). To a stirred solution of 22.5 g. of propylamine-1-C^{14} perchlorate in 20 ml. of 35% perchloric acid at 25° is added dropwise (2 hours) a solution of 21. g. of sodium nitrite in 30 ml. of water; then 25 ml. of distillate is collected. The distillate is acidified with hydrochloric acid, then redistilled (Note 4) and treated with potassium fluoride to salt out the alcohols which are separated. The crude product is diluted with 2.0 ml. of carrier 1-propanol and fractionated through a Podbielniak Micro Column. The yield of 1-propanol-1, 2-$C^{14}_{1/2}$ is 0.76 g., and the compound is assayed as the 3,5-dinitrobenzoate, m.p. 73°. Degradation studies (Note 5) indicate that 8.5% of the activity is located in a rearrangement position, presumed to be 2 (Note 6).

B. Notes

1. The propionitrile-1-C^{14} is hydrolyzed with 90% sulfuric acid, forming propionic-1-C^{14} acid in 67% yield. Reduction of the acid with lithium aluminum hydride gives 1-propanol-1-C^{14} in 50% yield. Degradation

studies (Note 5) indicate that there is no isotopic rearrangement. Propionitrile-1-C^{13} has been prepared[1] in 80% yield by heating 7 ml. of ethyl bromide with 3.2 g. of potassium cyanide-C^{13} and 15 ml. of triethylene glycol for 6 hours at 100° in a sealed tube. The yield of nitrile, isolated by vacuum distillation, was 7.0 g. (4.9 mmoles).

2. The procedure is adapted from the general method of Amundsen[2] for reducing nitriles to primary amines.

3. The synthesis is primarily a mechanism study, for the principal product is 2-propanol-1-C^{14}; yield 3.4 g. (41%), b.p. 80–84°. Whitmore[3] found that treating propylamine with nitrous acid gave 7% propyl alcohol, 32% isopropyl alcohol, 28% propylene and traces of ethers.

4. Whitmore[3] found that 5.3% of the starting amine was unreacted.

5. Directions are given by Roberts for oxidation of the 1-propanol-1, 2-$C^{14}_{1/2}$ with cold acid permanganate, forming propionic-1,2-$C^{14}_{1/2}$ acid, b.p. b.p. 135.5–139.5° (42%), which was assayed as the *p*-toluidide, m.p. 124°. Decarboxylation of the acid with hydrazoic acid formed carbon-C^{14} dioxide (73% yield) and ethylamine-1-C^{14}, which was assayed as the *N*-ethyl-1-C^{14} *p*-bromobenzenesulfonamide (30% yield), m.p. 80.5°.

6. Ethylamine-1-C^{14} under the same conditions gave[4] a 38% yield of ethanol-$C^{14}_{1/2}$ that was 1.5% isotopically rearranged; also see phenethyl-α, β-$C^{14}_{1/2}$ alcohol.

[1]C. D. Wagner, D. P. Stevenson and J. W. Otvos, J. Am. Chem. Soc., 72, 5786 (1950).

[2]L. H. Amundsen and L. S. Nelson, J. Am. Chem. Soc., 73, 282 (1951).

[3]F. C. Whitmore and R .S. Thorpe, J. Am. Chem. Soc., 63, 1118 (1941).

[4]J. D. Roberts and J. A. Yancey, J. Am. Chem. Soc., 74, 5943 (1952).

PROPYLAMINE-3-C^{14}

$$CH_3COCH_2COOC_2H_5 \xrightarrow[NaOC_2H_5]{C^*H_3I} CH_3COCHCOOC_2H_5 \xrightarrow[H_2SO_4]{NaOH}$$

$$\underset{C^*H_3}{\underset{|}{CH}}$$

(a)

$$CH_3COCH_2C^*H_3 \xrightarrow{(NH_4)_2SS_x} C^*H_3CH_2CH_2C^*ONH_2 \xrightarrow{Ba(OBr)_2}$$

(b) (c)

$$C^*H_3CH_2CH_2NH_2 + C^*O_2$$

(d)

E. Cerwonka, R. C. Anderson and E. V. Brown, J. Am. Chem. Soc., 75, 28 (1953).

A. Procedure

(a) *Ethyl 2-Methyl-C^{14}-acetoacetate* (Note 1). To a solution of 1.6 g. (70 mmoles) of sodium in absolute ethanol is added 9.37 g. (72 mmoles) of ethyl acetoacetate, under anhydrous conditions. To the stirred mixture, heated under reflux, is added dropwise a solution of 9.12 g. of methyl-C^{14} iodide in absolute alcohol (total volume 100 ml.). Heating and stirring are continued until the mixture tests neutral; then the mixture is cooled, and the solution is separated from any precipitated salt and distilled through a short fractionating column. The yield of crude product suitable for use is 6.6 g., b.p. 67–75° (12 mm.).

(b) *2-Butanone-4-C^{14}* (Note 2). The crude ethyl 2-methyl-C^{14}-acetoacetate is stirred at room temperature with 5% sodium hydroxide solution until hydrolysis and dissolution are complete (4 hours). The aqueous layer is separated, acidified with 50% sulfuric acid and heated slowly to boiling, one-half of the total volume being distilled. The distillate is made alkaline (Note 3) with solid sodium hydroxide and redistilled. The crude product is separated from the water and fractionated; yield 2.0 g. (40% based on methyl iodide), b.p. 78–80°.

(c) *Butyramide-1,4-$C^{14}_{1/2}$* (Note 4). The Willgerodt reaction is performed by the procedure of Cavalieri[1] for aliphatic ketones, which follows.

A mixture of 20 g. of sulfur, 1 g. of the ketone (in this case 2-butanone-4-C^{14}), 2 ml. of dioxane and 4 ml. of ammonium polysulfide is heated at 165–170° for 5 hours in a sealed, eight-inch, Pyrex ignition tube. The tube is cooled and opened, and the contents are washed into a beaker with concentrated ammonia and evaporated to dryness on a steam-bath. The residue is leached with 20 ml. of boiling water and again taken to dryness; the process is repeated with 5 ml. of boiling water. The product then is allowed to sublime upon the walls of the beaker where it is scraped off; yield 0.120 g. (10%), m.p. 116°. Degradation studies (see below) indicate that 60% of the isotope occurs at C–4.

(d) *Propylamine-3-C^{14} Hydrochloride* (Note 5). To a suspension of 0.33 mmoles of butyramide-1,4-$C^{14}_{1/2}$ in 6.0 ml. of 0.36 *N* barium hydroxide is added 0.36 mmoles of bromine by micropipette. The mixture is frozen with liquid nitrogen, and the flask is evacuated and closed off by stopcock. The mixture is warmed to room temperature and shaken, then heated on a hot water-bath (70°) for 3 or 4 minutes until the degradation take place, as evidenced by the precipitation of barium carbonate (Note 6). After cooling of the mixture, the vacuum is released and the barium carbonate-C^{14} is filtered off and washed with several portions of water (Note 7). The alkaline filtrate is distilled slowly in a microdistillation apparatus, and 2 drops of concentrated hydrochloric acid are added to the distillate, which then is evaporated to dryness under vacuum. The yield is 60%, m.p. 156°, and the molar specific activity is 60% of that of the starting material (Note 8).

B. Notes

1. The procedure is a modification of the method of Marvel and Hager.[2] The reaction is general, and yields of substituted acetoacetic esters are of the order of 70%.

2. The procedure is adapted from the method of Johnson and Hager[3] for the ketonic decarboxylation of a substituted acetoacetic ester.

3. The alkali removes any acids formed by the acid cleavage of the substituted acetoacetic ester, which is favored by strong alkali and higher temperature.

4. The preparation is incidental to a study of the mechanism of the Willgerodt reaction with aliphatic ketones. As butyramide would result from the migration of the functional group to either end of the chain in methyl ethyl ketone, the partial migration to both ends in the case of unsymmetrical normal alkyl ketones was revealed by labeling one end.

5. The procedure is a modification of the procedure of Brown, Cerwonka and Anderson;[4] see benzylamine-α-C^{14}. To give improved yields of aliphatic amine, the molar proportions of amide, bromine and barium hydroxide are altered to 1 : 1 . 1 : 3.

6. The precipitate may be coagulated by immersing the flask for a minute in boiling water.

7. The yield of barium carbonate is 70–75% in degradations of aliphatic amides. Samples are purified by reprecipitation before counting; the molar specific activity is 41% of that of the starting 2-butanone-4-C^{14}.

8. When a dialkyl ketone undergoes the Willgerodt[5,1] reaction, the functional group migrates to the structurally simple end of the molecule. The isotopic distribution found in the products from unsymmetrical di-*n*-alkyl ketones reveals an increasing preferential tendency towards migration to the shorter end of the chain as the carbon chain is lengthened.

C. Other Preparations

A similar series of compounds is prepared by the same procedures, starting with 2-pentanone-1-C^{14} and 2-heptanone-1-C^{14}, giving respectively: valeramide-1,5-$C^{14}_{1/2}$ (yield 25%, m.p. 108°, 68% isotope at C–1), butylamine-4-C^{14} hydrochloride (yield 60%, m.p. 193°; the molar specific activity is 32% of that of the valeramide) and heptanamide-1,7-$C^{14}_{1/2}$ (yield 30%, m.p. 95–96°, 86% isotope at C–1), hexylamine-6-C^{14} hydrochloride (yield 70%, m.p. 215°; the molar specific activity is 14% of that of the heptanamide).

[1]L. Cavalieri, D. B. Pattison and M. Carmack, J. Am. Chem. Soc., 67, 1783 (1945).

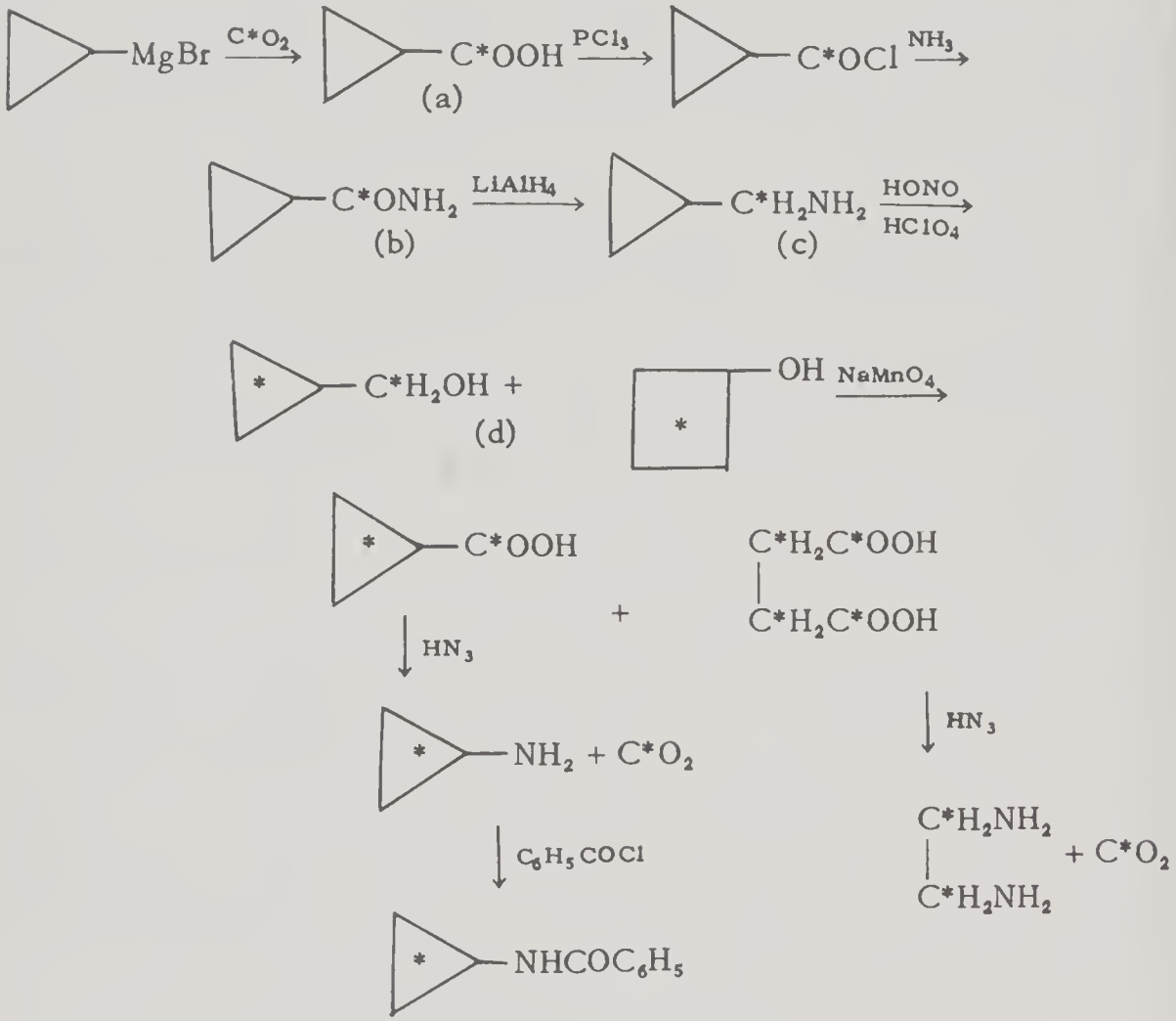
[2]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 248.

[3]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 351.

[4]E. V. Brown, E. Cerwonka and R. C. Anderson, J. Am. Chem. Soc., 73,3735 (1951).

[5]*Organic Reactions*, Vol. III, Wiley, New York, 1946, p. 83.

CYCLOPROPANEMETHYLAMINE-α-C^{14}



J. D. Roberts and R. H. Mazur, J. Am. Chem. Soc., 73, 3543, 2509 (1951).

A. Procedure (Note 1)

(a) *Cyclopropanecarboxylic-C^{14} Acid.* A Grignard reagent prepared from bromocyclopropane (Note 2) is carbonated with carbon-C^{14} dioxide. An ether extract[1] of the hydrolyzed reaction mixture is dried over Drierite, concentrated on a steam-bath and distilled at 94–95° (26 mm.); n_D^{20} 1.43901, m.p. 18–19° (Note 3).

(b) *Cyclopropanecarboxamide-C^{14}* (Note 4). A mixture of 3 moles of cyclopropanecarboxylic-C^{14} acid and 2 moles of phosphorus trichloride is refluxed at 80°; then the liquid is decanted from the phosphorous acid by washing with petroleum ether, and fractionated. The yield of cyclopropanecarbonyl-C^{14} chloride is quantitative.

The amide is prepared[1] by saturating a solution of 171 g. (1.64 moles) of the acid chloride in 1500 ml. of absolute ether at 9° with ammonia under anhydrous conditions. The mixture is evaporated to dryness under reduced pressure, and the residue is extracted with chloroform in a Soxhlet apparatus; yield 126.5 g. of colorless prisms (91%), m. p. 125°.

(c) *Cyclopropanemethylamine-α-C^{14}.* The amine is prepared by reduction of the amide with lithium aluminum hydride,[2] and may be isolated by steam distillation. Cyclopropanemethylamine-α-C^{14} hydrochloride, dried by evaporation with benzene and crystallized from absolute alcohol-ether, melts at 203–204° (Note 5).

(d) *Cyclopropanemethanol-$C^{14}_{1/4}$ and Cyclobutanol-$C^{14}_{1/4}$* (Note 6). Cyclopropanemethylamine-α-C^{14} is generated from 28 g. (0.26 mole) of the hydrochloride by shaking with an excess of freshly precipitated silver oxide (Note 7). The filtered aqueous solution is acidified with ice-cold *N* perchloric acid (Note 8) and treated with an ice-cold aqueous solution of sodium nitrite (0.8 mole). The mixture is steam-distilled, and the distillate is saturated with potassium carbonate and extracted with ether. The combined extract is dried over magnesium sulfate, concentrated and fractionated through a center-tube column.[3] The yield of cyclobutanol-$C^{14}_{1/4}$-cyclopropanemethanol-$C^{14}_{1/4}$ mixture is 6.4 g. (35%), b.p. 57° (42 mm.); n_D^{25} 1.4316 (Note 9). According to infrared analysis, the composition is: 47% cyclobutanol-$C^{14}_{1/4}$, 48% cyclopropanemethanol-$C^{14}_{1/4}$ and 5% C^{14}_1-3-butene-1-ol. Degradation by permanganate oxidation forms cyclopropanecarboxylic-$C^{14}_{1/4}$ acid and succinic-$C^{14}_{1/4}$ acid. Decarboxylation by the Curtius degradation[4] forms carbon-C^{14} dioxide possessing 45% and 36% of the activity of the respective acids, while the corresponding cyclopropylamine-$C^{14}_{1/3}$ and ethylenediamine-$C^{14}_{1/2}$ possess 54% and 64% of the activity of the respective acids.

B. Notes

1. As no experimental details are given for any of the isotopic preparations, the procedures are those from earlier nonisotopic syntheses.

2. Directions are given by Roberts[5] for preparing bromocyclopropane (58% yield) by the Hunsdiecker degradation of cyclopropanecarboxylic acid[6] by the action of bromine on the silver salt. The chloride is an exceptionally unreactive halide, refluxing for 3 days in the presence of catalytic iodine being necessary to form the Grignard reagent.

3. The acid may be prepared in 74–79% yield from 4-chlorobutyronitrile.[1,6]

4. The amide is prepared best through the acid chloride,[7] which could not be obtained through the use of thionyl chloride by Schlatter,[1] although the preparation has been reported by Jeffery,[8] b.p. 119–119.5° (763 mm.).

5. An analytical sample melted at 206–207.5°. The *N*-(cyclopropylmethyl)benzamide melted at 72.5–74.5°, following sublimation.

6. The preparation is incidental to a tracer study of the mechanism of the rearrangement reaction[9] in which cyclopropanemethylamine and nitrous acid give mixtures of cyclobutanol and cyclopropanemethanol. The interconversion of cyclopropylmethyl and cyclobutyl derivatives appears to proceed by irreversible carbonium ion reactions (not anion or free radical). In the case under study, the three methylene groups of the starting material attain a degree of equivalence as some intermediate, presumably the carbonium ion, generated by the general aliphatic primary amine-nitrous acid reaction in polar media. Essentially the same mixture of products is obtained starting with cyclobutylamine, the amounts of the various products appearing to correspond roughly with the relative carbonium ion stabilities.

Another rearrangement, characteristic of carbonium ion reactions, has been reported by Roberts;[10] see Roberts and Mazur[11] for directions (nonisotopic). Cyclopropanemethanol-α-C^{14} was converted to 4-chloro-1-butene-1,3,4-$C^{14}_{1/3}$ by the action of anhydrous zinc chloride-concentrated hydrochloric acid (Lucas reagent). Degradation studies showed that the isotope was distributed equally amonc C–1, C–3 and C–4, which were the original methylene carbon atoms. The halogen was replaced by hydrogen through the Grignard reaction; then the 1-butene-1,3,4-$C^{14}_{1/3}$ was hydroxylated with hydrogen peroxide and formic acid (see allyl-3-C^{14} *p*-tolyl ether, Note 7) to form 1,2-butanediol-1,3,4-$C^{14}_{1/3}$. Oxidation with sodium metaperiodate formed propionaldehyde-2,3-$C^{14}_{1/2}$, and formaldehyde-C^{14} having 33.7% of the activity of the starting material. Further oxidation of the propionaldehyde with permanganate, followed by decarboxylation (Curtius) of the propionic-2,3-$C^{14}_{1/2}$ acid, formed nonistopic carbon dioxide.

Another example[12,13,10] of extensive isotope-position rearrangement is the solvolysis[14] of *exo*-2-norcamphanyl-2,3-C^{14}_2 *p*-bromobenzenesulfonate in glacial acetic acid (also see *endo*-2-norcamphanyl-2,3-C^{14}_2 *p*-bromobenzenesulfonate).[10,13]

The solvolysis[15] of 1-methyl-C^{14}-propyl *p*-toluenesulfonate forms 1-methyl-$C^{14}_{1/1}$-propyl-3-$C^{14}_{1/1}$ acetate, having 9% of the isotope located at C–3.

7. The procedure is not completely satisfactory, for some silver chloride is bound as an amine complex and precipitated on acidification.

8. This acid was selected to avoid complications resulting from the formation of halides in the mechanism study.

9. Cyclopropanemethanol boils at 122–123°, n_D^{25} 1.4300; carbanilate, m.p. 76.6–77.0° (from benzene); 3,5-dinitrobenzoate, m.p. 101.2–101.4°

(from benzene-hexane). Cyclobutanol[16] boils at 125°, n_D^{25} 1.4347; cyclobutyl carbanilate, m.p. 130.6–131.2°.

[1]M. J. Schlatter, J. Am. Chem. Soc., *63*, 1733 (1941).
[2]*Organic Reactions*, Vol. VI, Wiley, New York, 1951, Chapter 10.
[3]E. A. Naragon and C. J. Lewis, Ind. Eng. Chem., Anal. Ed., *18*, 448 (1946).
[4]A. A. Benson and J. A. Bassham, J. Am. Chem. Soc., *70*, 3939 (1948).
[5]J. D. Roberts and V. C. Chambers, J. Am. Chem. Soc., *73*, 3176 (1951).
[6]*Organic Syntheses*, Vol. 24, Wiley, New York, 1944, p. 36.
[7]P. Lipp, J. Buchkremer and H. Seeles, Ann., *499*, 1 (1932).
[8]G. H. Jeffery and A. I. Vogel, J. Chem. Soc., *1948*, 1804.
[9]N. J. Demjanow, Ber., *66*, 4393, 4961 (1907).
[10]J. D. Roberts, Atomic Energy Commission Report, NP-3437; Nuc. Sci. Abstracts, *5*, 7047 (1951).
[11]J. D. Roberts and R. H. Mazur, J. Am. Chem. Soc., *73*, 2509 (1951).
[12]J. D. Roberts and C. C. Lee, J. Am. Chem. Soc., *73*, 5009 (1951).
[13]J. D. Roberts, Atomic Energy Commission Report, NP-3391; Nuc. Sci. Abstracts, *5*, 6708 (1951).
[14]J. D. Roberts and V. C. Chambers, J. Am. Chem. Soc., *73*, 5034 (1951); J. D. Roberts, W. Bennett and R. Armstrong, J. Am. Chem. Soc., *72*, 3329 (1950).
[15]J. D. Roberts, W. Bennett, R. E. McMahon and E. W. Holroyd, Jr., J. Am. Chem. Soc., *74*, 4283 (1952).
[16]J. D. Roberts and C. W. Sauer, J. Am. Chem. Soc., *71*, 3925 (1949).

PUTRESCINE-1,4-C_2^{14}

$$NC^*(CH_2)_2C^*N \xrightarrow[Ni]{H_2} NH_2C^*H_2(CH_2)_2C^*H_2NH_2 \cdot 2HCl$$

D. G. M. Diaper, S. Kirkwood and L. Marion, Can. J. Chem., *29*, 964 (1951).

A. Procedure

Putrescine-1,4-C_2^{14} Dihydrochloride, (*1,4-Butanediamine-1,4-C_2^{14} Dihydrochloride*) (Note 1). Crude succinonitrile-1,4-C_2^{14} (0.222 g.), mixed with 2 ml. of ethanol and an equal volume of liquid ammonia, is sealed in a bomb and hydrogenated over Raney nickel catalyst at 100° and 1200 p.s.i. (Note 2). The mixture is diluted with 500 ml. of dilute hydrochloric acid and filtered; then the product is precipitated as the reineckate by treating the filtrate with an aqueous solution of ammonium reineckate[1] (Note 3); yield 0.362 g. The product is isolated as the hydrochloride by heating the reineckate with strong potassium hydroxide solution and trapping the vapors in dilute hydrochloric acid solution, which then is evaporated to dryness.

B. Notes

1. The procedure is adapted from the method of Arbuzov.[2]

2. The hydrogenation of 1.02 g. of *N*-(3-cyanopropyl)phthalimide, mixed with 3 ml. of ethanol and 1.0 ml. of ammonia (sp. gr., 0.88), at 100° and 1500 p.s.i. over Raney nickel gives a 91% yield of putrescine. Reduction with sodium and ethanol, by the method of Keil,[3] gives poor yields. The catalytic hydrogenation of nitriles yields a mixture of primary and secondary amines, but the proportion of primary amine is increased by the presence of ammonia, which greatly diminishes the reaction between primary amine and the intermediate aldimine, which accounts for the formation of secondary amine by loss of ammonia.

3. Putrescine reineckate is precipitated quantitatively, even in the presence of 60 g. of ammonium chloride per liter. The salt is soluble in acetone.

[1]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 555.

[2]B. A. Arbuzov and E. A. Pozhil'tsova, Bull. acad. sci. U.R.S.S., Classe sci. chim., 65 (1946); Chem. Abstracts, *42*, 5848 (1948).

[3]W. Keil, Ber., *59*, 2816 (1926).

DODECYLAMINE-1-C^{14}

$$CH_3(CH_2)_{10}C^*OOH \xrightarrow[Al_2O_3]{NH_3} \underset{(a)}{CH_3(CH_2)_{10}C^*N} \xrightarrow[n\text{-BuOH}]{[H]\ Na} \underset{(b)}{CH_3(CH_2)_{10}C^*H_2NH_2}$$

H. J. Harwood and A. W. Ralston, J. Org. Chem., *12*, 740 (1947).

A. Procedure

(a) *Dodecanenitrile-1-C^{14}*,(*Lauronitrile-C^{14}*) (Note 1). Lauric-C^{14} acid (6.73 g.) is converted to the nitrile by passage over 10 g. of aluminum oxide catalyst at 390° in a stream of ammonia (Note 2). The ammonia rate is 200 cc. per minute, and the acid is vaporized by being dropped into a flask at 320–340°, a 3.23-g. portion of nonisotopic lauric acid being used as a chaser. A total time of 130 minutes is required for the reaction, and the apparatus is then swept with ammonia for an additional hour. The product is collected in petroleum ether, washed with water and dried over potassium carbonate. Removal of solvent leaves 8.82 g. (97.4%) of crude product.

(b) *Dodecylamine-1-C^{14}*. Lauronitrile-C^{14} (8.82 g., crude) in 19 g. of anhydrous 1-butanol is added over 10 minutes to a vigorously stirred, boiling mixture of 6 g. of finely divided sodium and 75 ml. of toluene. After an additional 25 ml. of toluene and 10 ml. of butanol are added, the mixture is stirred and heated until all the metal has reacted. Water is added, and the toluene layer is separated and dried with potassium car-

bonate. After removal of solvent, the crude product is dissolved in 100 ml. of 50% alcohol containing 4 ml. of concentrated hydrochloric acid The solution is extracted with four portions of petroleum ether to remove unreacted nitrile. The acid solution is heated to remove most of the alcohol, and then made alkaline. The product is again extracted into petroleum ether and dried over potassium carbonate. Evaporation of the solvent gives a yield of 8.25 g., 91.5% (Note 3).

B. Notes

1. The procedure is an adaptation of the method of Hoerr[1] for preparing nitriles from the corresponding fatty acid.

2. The catalyst chamber is 22 × 1.6 cm. and contains a 7.25-mm. (o.d.) thermocouple well.

3. The acetate is prepared by adding 2.5 ml. of glacial acetic acid to a solution of the amine in 50 ml. of benzene, and crystallizing at 15°. The yield, after recrystallization from benzene at 15°, is 9.5 g., m.p. 68.5–69.0°.

[1]C. W. Hoerr, E. F. Binkerd, W. O. Pool and A. W. Ralston, J. Org. Chem., 9, 68 (1944); A. W. Ralston, W. M. Selby and W. O. Pool, Ind. Eng. Chem., 33, 682 (1941).

BENZYLAMINE-α-C^{14} HYDROCHLORIDE

$$C_6H_5C^*OCH_3 \xrightarrow[C_5H_5N]{NH_4OH,\ S} \underset{(a)}{C_6H_5C^*H_2CONH_2} \xrightarrow[(HCl)]{Ba(OBr)_2} \underset{(b)}{C_6H_5C^*H_2NH_2 \cdot HCl}$$

$$+$$

$$C_6H_5C^*H_2COONH_4 \xrightarrow{HCl} \underset{(c)}{C_6H_5C^*H_2COOH}$$

E. V. Brown, E. Cerwonka and R. C. Anderson, J. Am. Chem. Soc., 73, 3735 (1951).

A. Procedure

(a) *2-Phenylacetamide-2-C^{14}* (Note 1). Five sealed Carius tubes, each containing 3.75 g. of sulfur, 2.5 g. of acetophenone-1-C^{14}, 3.0 ml. of pyridine and 5.0 ml. of 15 *N* ammonium hydroxide (Note 2), are heated for 4 hours in a furnace at 165°. The cooled tubes are opened (Note 3), and the contents are washed into one beaker with concentrated ammonium hydroxide, and evaporated to dryness on a steam-bath to remove ammonium sulfide. The residue is ground in a mortar, extracted with several portions of boiling water (total 200 ml.) and decolorized with carbon, and the

solution is cooled to crystallize the product; yield 10 g. A second crop (1.2 g.) is obtained by evaporating the mother liquor to half-volume. The mother liquor is made alkaline with sodium carbonate (Note 4) and extracted with five portions of ether, yielding 0.5–1.0 g. of product. Purification is accomplished by recrystallization from water; yield 80%, m.p. 156.5–157.5°. Degradation studies (see below) indicate that there is no isotopic dilution or rearrangement.

(b) *Benzylamine-α-C^{14} Hydrochloride* (Note 5). To a suspension of 0.045 g. (0.33 mmole) of 2-phenylacetamide-2-C^{14} in 6.0 ml. of 0.36 *N* barium hydroxide (Note 6) is added 0.67 mmole of bromine by micropipette. The mixture is frozen with liquid nitrogen, and the flask is evacuated and closed by stopcock. The mixture is warmed to room temperature, shaken to effect solution in the hypobromite and heated for 3–4 minutes in a water-bath to effect the degradation; then the flask is heated in boiling water for a minute to coagulate the precipitate. After cooling, the nonisotopic barium carbonate is collected, washed with water and dried (Note 7). The combined alkaline filtrate and washings are distilled slowly in a microdistillation apparatus with an indented column, the oily benzylamine-α-C^{14} being collected with the first few ml. of water. The distillate is acidified with 2 drops of concentrated hydrochloric acid, and the salt solution is evaporated to dryness under vacuum; yield 70–80%. The product melts at 256°, after recrystallization from butanol, and the molar specific activity is the same as that of the starting ketone.

(c) *Phenylacetic-2-C^{14} Acid.* The alkaline solution remaining after the final extraction of the amide is acidified with hydrochloric acid and extracted with five portions of ether. Evaporation gives 0.5 g. of crude, oily product which is crystallized by dissolution in sodium carbonate solution, followed by slow acidification; yield 2% based on acetophenone. The melting point is 76.5–77.5°, after recrystallization from petroleum ether, and the molar specific activity is the same as that of the starting ketone. Degradation studies indicate that there is no isotopic rearrangement (Note 8).

B. Notes

1. The synthesis is incidental to a study of the mechanism of the Willgerodt reaction.[1] The procedure is adapted from that described by DeTar and Carmack.[1,2] King[3] has established that the carbonyl group need not be adjacent to the aromatic ring, and the reaction proceeds with alcohols, olefins, acetylenes and aldehydes, as well.[4]

2. DeTar and Carmack[2] have determined that the optimum yield is obtained when: the mole ratio of sulfur to ketone is about 10, the ammonium hydroxide is 12–15 *N*, the mixture is heated at 160–190° for 4–6 hours, and the organic solvent is pyridine or dioxane. The combined yield of

phenylacetamide-phenylacetic acid is 85.6%, compared to 63% by the original procedure of Willgerodt and Merk.[1]

3. No pressure is present at room temperature.

4. The solution is pH 5, presumably due to decomposition of ammonium phenylacetate during the evaporation.

5. The procedure is an adaptation of that of Hoogewerf,[5] who reported an 80–85% yield of benzylamine, b.p. 182–184°.

6. Barium hydroxide, rather than the usual sodium hydroxide, is employed to eliminate the carbonate blank normally present in the latter.

7. The barium carbonate (yield 100% based on 2-phenylacetamide) is reprecipitated before assay.

8. Directions are given by Brown for converting the acid to 2-phenylacetamide-2-C^{14} (75% yield) by reaction with thionyl chloride, followed by treatment with ammonium hydroxide. Degradation in the manner described gives nonisotopic barium carbonate and benzylamine-α-C^{14} hydrochloride having the same molar specific activity as the amide, acid and ketone.

C. Other Preparations

2-Phenylacetamide-1-C^{14} and phenylacetic-1-C^{14} acid are prepared in the same manner, starting with acetophenone-2-C^{14}. Degradation studies again indicate that there is no isotopic rearrangement.

The preparation of 2-phenylacetamide-2-C^{14} (62% yield, m.p. 159–160°; 80% yield at the 10-mmole level) and phenylacetic-2-C^{14} acid (1.5% yield, m.p. 75–76°) at the 265-mmole scale, and the preparation of benzylamine-α-C^{14} hydrochloride (45% yield) at the 1-mmole scale, by essentially the procedures given, have been described by Dauben.[6] An ambiguous degradation by decarboxylation over copper chromite (see phenylacetic-2-C^{14} acid, Note 3) appeared to indicate that a little activity was found at C–1. The carbon dioxide obtained from the amide by the Hofmann reaction contained only 0.1% of the initial activity. Experiment has shown[6] that when equimolar amounts of phenylacetic-1-C^{14} acid and 2-phenylacetamide are heated together under the conditions of the Willgerodt reaction, 2-phenylacetamide-1-C^{14} is formed by exchange in about 10% yield.

The preparation of phenylacetic-2-C^{13} acid from acetophenone-1-C^{13} by the Kindler-Willgerodt reaction (morpholine), followed by alkaline hydrolysis of the thiomorpholide, has been described by Schantz.[7] Degradation by decarboxylation over iron filings at 250° gave rise to nonisotopic carbon dioxide. The same preparation for the C^{14}-compound has been reported by Dauben.[6]

The preparation of benzylamine-α-C^{14} hydrochloride (34–47% yield) has been reported by Douglas.[8] Sodium benzoate-C^{14} was reacted with cyanogen bromide at 280–300°, and the benzonitrile-C^{14} formed was re-

duced with lithium aluminum hydride. The molar specific activity of the benzoic-C^{14} acid formed by hydrolysis of the nitrile was 72–87% of that of the starting material, while that of the amine was 83.2%.

[1]*Organic Reactions*, Vol. III, New York, 1946, Chapter 2, p. 97.
[2]D. F. DeTar and M. Carmack, J. Am. Chem. Soc., *68*, 2025, 2029 (1946).
[3]J. A. King and F. H. McMillan, J. Am. Chem. Soc., *68*, 525, 632 (1946).
[4]*Organic Reactions*, Vol. III, Wiley, New York, 1946, p. 83.
[5]S. Hoogewerf and W. A. van Dorp, Rec. trav. chim., *5*, 251 (1886).
[6]W. G. Dauben, J. C. Reid, P. E. Yankwich and M. Calvin, J. Am. Chem. Soc., *72*, 121 (1950); *68*, 2117 (1946); M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert, and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 196.
[7]E. M. Schantz and D. Rittenberg, J. Am. Chem. Soc., *68*, 2109 (1946).
[8]D. E. Douglas, J. Eccles and A. E. Almond, Can. J. Chem., *31*, 1127 (1953).

α-METHYLPHENETHYLAMINE-β-C^{14} SULFATE
(C_1^{14}-Amphetamine Sulfate)

$$C_6H_5C^*H_2COOH \xrightarrow{SOCl_2} C_6H_5C^*H_2COCl \xrightarrow{C_2H_5OMgCH(COOC_2H_5)_2}$$

$$C_6H_5C^*H_2\overset{\overset{O}{\|}}{C}CH(COOC_2H_5)_2 \xrightarrow[H_2SO_4]{HOAc} C_6H_5C^*H_2COCH_3 \xrightarrow[NaOH]{NH_2OH\cdot HCl}$$

(a) (b)

$$C_6H_5C^*H_2\overset{\overset{NOH}{\|}}{C}CH_3 \xrightarrow[Ni]{H_2} C_6H_5C^*H_2\overset{\overset{NH_2}{|}}{C}HCH_3 \cdot 1/2\ H_2SO_4$$

(c) (d)

J. W. Wilson III, J. Am. Pharm. Assoc., Sci. Ed., 39, 687 (1950).

A. Procedure (Note 1)

(a) *Ethyl (Phenylacetyl-2-C^{14})malonate* (Note 2). A mixture of 0.544 g. (4 mmoles) of phenylacetic-2-C^{14} acid and 2.9 ml. of thionyl chloride is refluxed for 2 hours. The excess reagent is distilled under vacuum, after which dry benzene and toluene are successively added and vacuum-distilled. The phenylacetyl-2-C^{14} chloride is refluxed for 1.5 hours with 10 ml. of ether containing a 10% excess of ethyl ethoxymagnesiummalonate (Note 3). The solution is cooled to 5° and hydrolyzed by the slow addition of 10 ml. of 10% sulfuric acid, and the product is isolated by continuous ether extraction, followed by solvent evaporation.

(b) *1-Phenyl-2-propanone-1-C^{14}* (Note 2). The crude ketoester is hydrolyzed and decarboxylated by refluxing for 5 hours with a mixture of 4.5 ml. of glacial acetic acid, 1.5 ml. of water and 0.5 ml. of sulfuric acid. The ice-cooled mixture is basified with 12 ml. of 20% sodium hydroxide

and 20 ml. of water, then continuously extracted with ether; evaporation of the solvent leaves the product as a residue (Note 4).

(c) *1-Phenyl-2-propanone-1-C^{14} Oxime* (Note 5). To a vigorously stirred mixture of the crude 1-phenyl-2-propanone-1-C^{14}, 0.300 g. of hydroxylamine hydrochloride and 3 ml. of water at 40° is added 2.2 ml. of 4.02 *N* sodium hydroxide over a period of 20 minutes. The mixture is stirred for 30 minutes, followed by repetition of the addition of hydroxylamine hychloride and sodium hydroxide. The mixture is neutralized with glacial acetic acid (0.6 ml.), and the product is continuously extracted with ether and concentrated by evaporation.

(d) *α-Methylphenethylamine-β-C^{14} Sulfate.* The crude oxime is hydrogenated at atmospheric pressure over Raney nickel at room temperature in 7 ml. of stirred alcoholic solution. The hydrogen uptake is complete in 4 hours and corresponds to 1.86 mmoles of oxime. The catalyst is removed by centrifuging and washed with three 3-ml. portions of ether. The combined solution is neutralized with 0.43 ml. of 3.42 *N* sulfuric acid and cooled to −15°. The product is collected by centrifuging, washed with three 5-ml. portions of ether and dried under vacuum; yield 0.279 g., 38% based on phenylacetic acid (Note 6).

B. Notes

1. The synthesis is carried out without isolating the intermediates.

2. The procedure is adapted from the method of Walker and Hauser.[1] The method is excellent for preparing higher aliphatic or aliphatic-aromatic methyl ketones and certain substituted acetophenones.

3. To 5.35 g. (0.22 mole) of magnesium, in a 500-ml. three-necked flask equipped with a mercury-sealed stirrer, dropping funnel, reflux condenser and drying tube, is added 5 ml. of absolute ethanol and 0.5 ml. of carbon tetrachloride. When the vigorous reaction has proceeded for a few minutes, 75 ml. of absolute ether is cautiously added, followed by a solution of 35.2 g. (0.22 mole) of ethyl malonate, 20 ml. of absolute ethanol and 25 ml. of ether, while rapid refluxing is maintained. Heating is continued until the metal is dissolved (3 hours). A suitable aliquot is added to an ethereal solution of the acid chloride.

4. Walker and Hauser[1] report a yield of 71% (based on the acid chloride), b.p. 97–98.5° (13 mm.); semicarbazone, m.p. 187–190°.

5. The procedure follows the method of Hey.[2] The oxime melts at 70°.

6. The *N*-benzoyl derivative melts at 132–134°. The method is a modification of the method of Hey[2]. The hydrochloride is very hygroscopic, m.p. 145–147°.

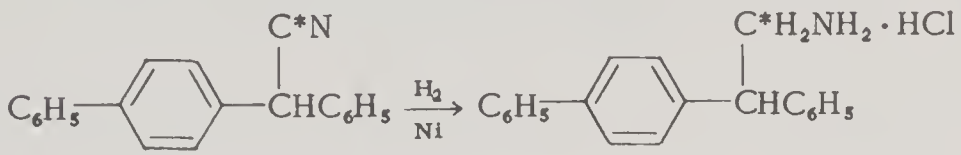
C. Other Preparations

α-Methyl-*p*-(1-methylethoxy)benzylamine-α-C^{14} [yield 84%, over-all yield 40% based on barium carbonate, b.p. 150–170° (bath temperature)

at 20 mm.] and *p*-cylohexyloxy-α-methylbenzylamine-α-C^{14} [yield 73%, b.p. 175–180° (bath temperature) at 16 mm.; over-all yield 40% based on barium carbonate] have been prepared by Brierley and McCoubrey (see aryl C^{14}-ketones, Method VII) by reduction of the oximes in 90% methanol with the addition of 3% sodium amalgam and acetic acid in small portions. The corresponding hydrochlorides melt at 180° and 122°, respectively.

[1]H .G. Walker and C. R. Hauser, J. Am. Chem. Soc., 68, 1386 (1946).
[2]D. H. Hey, J. Chem. Soc., *1930*, 18.

p,β-DIPHENYLPHENETHYLAMINE-α-C^{14} HYDROCHLORIDE
(β-Phenyl-4-biphenylethylamine-α-C^{14} Hydrochloride)



L. S. Ciereszko and J. G. Burr, Jr., J. Am. Chem. Soc., 74, 5431 (1952).

A. Procedure

(4-Biphenylyl)phenylacetonitrile-1-C^{14} (3.0 g., 11.5 mmoles) is reduced in alcohol solution with hydrogen over an alkaline (briefly washed) W-7 Raney nickel catalyst in a Parr apparatus. The hydrogen uptake (15 mmoles, 65%) is complete in 15 hours (Note 1). The catalyst is filtered off, and the filtrate is evaporated. The residue is stirred with 50 ml. of 20% hydrochloric acid, and the resulting salt is collected, dissolved in alcohol and precipitated by the addition of ether. The yield of colorless crystalline solid is 1.90 g., m.p. 252°. Reworking the mother liquor raises the yield to 2.40 g., 70% (Note 2).

B. Notes

1. Some of the difficultly soluble nitrile remains at the end.

2. Recrystallization of the difficultly soluble hydrochloride from water does not change the melting point; picrate, m.p. 220°; free base, m.p. 90°.

C. Other Preparations

A series of 2,2-diarylethylamine-1-C^{14} compounds is prepared by the same method:

p-Methyl-β-phenylphenethylamine-α-C^{14} (68%, oil); picrate, m.p. 203°; hydrochloride, m.p. 236°. The preparation of the water-insoluble *p*-methyl-β-phenylphenethylamine-α-C^{14} nitrite is described by Bailey.[1]

m-Methyl-β-phenylphenethylamine-α-C^{14} (86%, oil); hydrochloride, m.p. 245°; picrate, m.p. 196°.

o-Methyl-β-phenylphenethylamine-α-C^{14} hydrochloride; yield[1] 2.7 g. (m.p. 266°) from 3.00 g. of the nitrile; picrate, m.p. 244°.

p-Methoxy-β-phenylphenethylamine-α-C^{14} (23%, oil); hydrochloride, m.p. 195°; picrate, m.p. 172°.

2,4,6-Trimethyl-β-phenylphenethylamine-α-C^{14} hydrochloride; yield[1] 13.6 g. (m.p. 259°) from 17 g. of the nitrile; picrate, m.p. 249°. The water-insoluble nitrite is remarkably stable for a salt of this nature, m.p. 140°.

3,4-Dimethyl-β-phenylphenethylamine-α-C^{14} hydrochloride, m.p. 236°; picrate, m.p. 186°; nitrite, m.p. 124° (dec.), decomposing in the presence of excess sodium nitrite.[1]

[1]P. S. Bailey and J. G. Burr, Jr., J. Am. Chem. Soc., 75, 2951 (1953).

N,N-DIMETHYL-4,4-DIPHENYL-3-BUTENYLAMINE-4-C^{14} HYDROCHLORIDE

$$(C_6H_5)_2C^*O \xrightarrow{(CH_3)_2NCH_2CH_2CH_2MgCl} (C_6H_5)_2C^*(OH)CH_2CH_2CH_2N(CH_3)_2 \xrightarrow{HCl}$$

(a)

$$(C_6H_5)_2C^* = CHCH_2CH_2N(CH_3)_2 \cdot HCl$$

(b)

T. A. Geissman, Atomic Energy Commission Report, UCRL-1233; Nuc. Sci-Abstracts, 5, 4722 (1951).

A. Procedure (Note 1)

(a) *4-Dimethylamino-1,1-diphenyl-1-butanol-1-*C^{14}. To 1.0 g. of preactivated magnesium (Note 2) under nitrogen is added slowly with stirring a solution of 5 ml. of freshly distilled 3-chloro-*N,N*-dimethylpropylamine (Note 3) in 30 ml. of absolute ether. The mixture is refluxed for 5 hours, 2.14 g. of benzophenone-C^{14} in 20 ml. of absolute ether is added to the suspension, and refluxing is continued for 4 hours. The cooled mixture is poured into ice-ammonium chloride solution, ammonium hydroxide is added, and the hydrolysate is extracted with ether. The ether so-

lution is extracted with 2 *N* hydrochloric acid (Note 4), and the aqueous solution is poured into cold dilure ammonium hydroxide. The product is collected, washed and dried; yield 2.43 g. (77%), m.p. 118–120°.

(b) N,N-*Dimethyl-4,4-diphenyl-3-butenylamine-4-*C^{14} *Hydrochloride.* A solution of the amino alcohol in 20 ml. of *N* ethanolic hydrogen chloride is refluxed for 1.5 hours, then diluted with 100 ml. of absolute ether and stored at −20° for 1 hour. The crystalline product is collected and recrystallized from absolute ethanol-ether; yield 2.24 g. (Note 5).

B. Notes

1. The procedure is an adaptation of the method described by Marxer.[1] Grignard reagents containing amino groups may be prepared, provided the halogen and amine groups are sufficiently distant and specified conditions are observed.

2. To a stirred mixture of 1.0 g. of magnesium and 10 ml. of absolute ether under dry nitrogen is added 0.2 ml. of bromobenzene. When the reaction has started with vigor, the solution is removed by pipette and the still wet, activated magnesium is used directly. The reaction of 3-chloro-*N*,*N*-dimethylpropylamine with magnesium, under the usual Grignard conditions, is erratic. Aiding the reaction, by the concurrent reaction of ethyl bromide with the magnesium, would introduce an undesirable second Grignard reagent into a small-scale isotopic synthesis.

3. Directions are given by Marxer[1] for preparing the starting material from dimethylamine and 1-bromo-3-chloropropane; also see Hass[2] and Gibbs.[3] The polymerization of these halogenated amines has been studied extensively by Marvel and co-workers.[3]

4. Sufficient water is added to dissolve the hydrochloride.

5. The combined mother liquor yields about 0.15 g. of the methiodide, upon treatment of the free base with ethereal methyl iodide.

[1] A. Marxer, Helv. Chim. Acta, *24*, 209E (1941).
[2] H. B. Hass and H. C. Huffman, J. Am. Chem. Soc., *63*, 1233 (1941).
[3] C. F. Gibbs, E. R. Littmann and C. S. Marvel, J. Am. Chem. Soc., *55*, 753 (1933).

2,2′-DICHLORO-*N*-METHYL-C^{14}-DIETHYLAMINE HYDROCHLORIDE (C_1^{14}-Nitrogen Mustard)

$$NH(CH_2CH_2OH)_2 \xrightarrow[(KOH)]{C^*H_3Br} C^*H_3N(CH_2CH_2OH)_2 \xrightarrow{SOCl_2} C^*H_3N(CH_2CH_2Cl)_2 \cdot HCl$$

W. W. Foreman, A. Murray III, and A. R. Ronzio, J. Org. Chem., *15*, 119 (1950).

A. Procedure

To an outgassed solution of 2.1065 g. (20.04 mmoles) of diethanolamine in 15 ml. of absolute alcohol, frozen with liquid nitrogen in a 25-ml. reaction flask containing a glass-enclosed magnetic stirring bar, is vacuum-transferred the methyl-C^{14} bromide prepared from 3.86 mmoles of methanol-C^{14} (Note 1). The flask is closed by stopcock, the stirrer is set for maximum mixing, and the mixture is melted as quickly as possible and stirred at room temperature for 42 hours. The mixture is concentrated by distilling 25% of the solvent (Note 2), then cooled and treated with a slight excess of potassium hydroxide in absolute alcohol. After being stirred for 30 minutes at 0°, the precipitated potassium bromide is filtered off, washed with absolute alcohol and dried (Note 3).

The combined filtered solution of *N*-methyl-C^{14}-2,2′-iminodiethanol (*N*-methyl-C^{14}-diethanolamine), is saturated with dry hydrogen chloride, concentrated to small volume with an air-stream, filtered (if the volume of potassium chloride is excessive) and evaporated to dryness in a 60-ml. flask. Benzene (25 ml.) is added and the mixture is saturated with hydrogen chloride. To the stirred mixture is added 5 ml. of purified thionyl chloride (Note 4) in small portions through the reflux condenser; then the mixture is heated at 60° under reflux for 3.5 hours with continuous stirring (Note 5). An additional 2 ml. of thionyl chloride is added in 0.5 ml. portions when hydrogen chloride evolution ceases. The excess thionyl chloride is destroyed by the dropwise addition of the calculated amount (1.4 ml.) of absolute methanol with cooling (Note 6). The mixture is diluted with 20 ml. of benzene, then cooled with an ice-bath, and the mixture of hydrochlorides is collected on a sintered-glass funnel and washed with several 5-ml. portions of cold benzene. The crystalline mass is transferred to the original flask and stirred briefly with 30 ml. of boiling chloroform; then the stirred mixture is allowed to cool for 15 minutes and is filtered. After four extractions in this manner (Note 7), the combined filtrate is evaporated at room temperature. The residue is transferred to a liquid-liquid extractor with 9 ml. of water and 1 ml. of concentrated hydrochloric acid. The solution is cooled with an ice-bath and treated slowly with a concentrated aqueous solution containing 0.4 g. of sodium nitrite. After 1 hour, the nitrosamine (Note 8) is removed by continuous ether extraction for 7 hours. The nearly colorless aqueous solution is evaporated with an air stream and dried in a desiccator over phosphorus pentoxide. The product is separated from sodium chloride by extraction with six portions of boiling chloroform. The residue, obtained by evaporation of the solvent, is dissolved in 10 ml. of dry acetone, decolorized with carbon and crystallized at −25°. The mother liquor is removed with a filter stick, and the crystals are washed with several 10-ml. portions of absolute ether and recrystallized from 17 ml. of acetone at

−40°. The yield from two runs, without working up mother liquors, is 0.6995 g. (52.5% based on 6.93 mmoles of methanol), m.p. 109–110° (Note 9).

B. Notes

1. Methyl iodide is not satisfactory in this reaction. When stoichiometric amounts of amine and halide are used, 20–35% of the amine is converted to the quaternary salt. A ratio of 5 moles of amine to 1 mole of methyl bromide, however, gives no quaternization. An apparatus diagram is given by Foreman.

2. The distillate contains no halogen.

3. The recovery of potassium bromide is 0.4376 g. (95.5% based on methanol).

4. The amount of thionyl chloride is based on the methylated and unmethylated diethanolamine and residual ethanol. Thionyl chloride (50 ml.) is purified by distillation, with the use of a fractionating column and a calcium chloride drying tube, first from quinoline (10 ml.), then from boiled linseed oil (20 ml.).

5. The opalescent mixture becomes successively opaque, gummy and crystalline.

6. An excess of methanol is avoided as it exerts some solvent action on the hydrochlorides.

7. The residual 2,2′-dichloroethylamine hydrochloride (2.2149 g.), m.p. 209–212.5°, is equivalent to 76.9% of the excess diethanolamine. A purified sample melts at 214–215°.

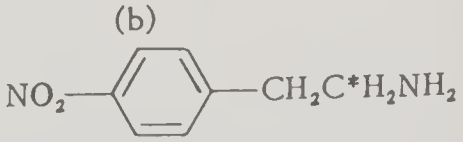
8. No satisfactory separation of the tertiary and secondary amines could be achieved through acetylation, benzoylation, the Hinsberg reaction, the isocyanates, fractional recrystallization or sublimation.

9. The product from a trial run yielded a chloroaurate melting at 82–84°. 2,2′-Dichloro-*N*-methyldiethylamine hydrochloride is volatile with boiling benzene, and hydrolyzes in neutral aqueous solution.

p-NITROPHENETHYLAMINE-α-C^{14}
[2-(*p*-Nitrophenyl)ethylamine-1-C^{14}]

$$C_6H_5CH_2Cl \xrightarrow{NaC^*N} C_6H_5CH_2C^*N \xrightarrow[C_2H_5OH,\ Na]{[H]} C_6H_5CH_2C^*H_2NH_2 \xrightarrow[H_2SO_4]{HNO_3}$$

(a) (b)



(c)

J. D. Roberts and C. M. Regan, J. Am. Chem. Soc., 75, 2069 (1953).

A. Procedure

(a) *Phenylacetonitrile-1-C^{14}*. Benzyl chloride is reacted with sodium cyanide-C^{14} according to the procedure of Adams and Thal.[1] The yield is 72%, b.p. 100–101° (8.5 mm.).

(b) *Phenethylamine-α-C^{14}*. To a solution of 12.1 g. of phenylacetonitrile-1-C^{14} in 100 ml. of absolute alcohol is added 11 g. of sodium. The mixture is refluxed until solution is complete (1.5 hours); then water (50 ml.) is added and the alcohol is distilled. The residue is extracted with ether, and the extract is dried and fractionally distilled; yield 4.1 g. (33%), b.p. 72–75° (8 mm.). The hydrochloride melts at 219–219.6°, after crystallization from a mixture of ether and absolute alcohol.

(c) p-*Nitrophenethylamine-α-C^{14}*. Phenethylamine-α-C^{14} is nitrated in sulfuric acid solution according to the procedure of Ehrlich and Pistschimuka,[2] which follows. The yield of hydrochloride is 39%, m.p. 212–214°.

To 100 g. of stirred, concentrated sulfuric acid is slowly added 30 g. of phenethylamine dropwise with cooling. The mixture is cooled to −10° and gradually treated with 11.1 ml. of nitric acid (d 1.5), then stored at room temperature for 15 hours. The reddish-brown liquid is poured onto crushed ice, basified with excess sodium hydroxide solution and extracted with ether. The ether solution is dried over sodium sulfate and evaporated to a basic, brownish oil. The crude base is converted to the hydrochloride by treatment with an excess of dilute hydrochloric acid and evaporation to dryness on a water-bath; yield 23 g., m.p. 211° after recrystallization from 96% alcohol.

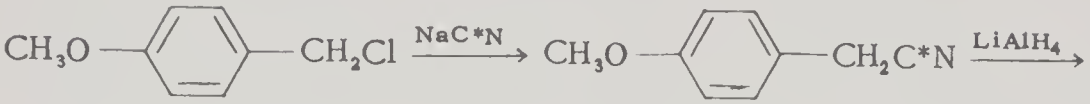
C. Other Preparations

The preparation of phenylacetonitrile-1-C^{13} by this method has been reported by Craig.[3]

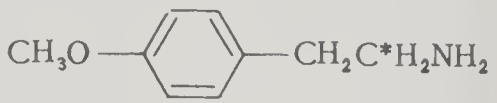
The preparation of phenylacetonitrile-1-C^{14} and phenethylamine-α-C^{14} by this method at the millimole-level has been described by Block.[4] A diagram of the microdistillation apparatus is given, and the final product (15.4% yield) was shown to be homogeneous by means of paper chromatography and paper electrophoresis.

[1] *Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 107.
[2] F. Ehrlich and P. Pistschimuka, Ber., *45*, 2428 (1912).
[3] J. T. Craig, J. B. Tindall and M. Senkus, Anal. Chem., *23*, 332 (1951).
[4] W. Block, Z. Naturforsch., *8B*, 440 (1953).

p-METHOXYPHENETHYLAMINE-α-C^{14}



(a)



(b)

J. D. Roberts and C. M. Regan, J. Am. Chem. Soc., 75, 2069 (1953).

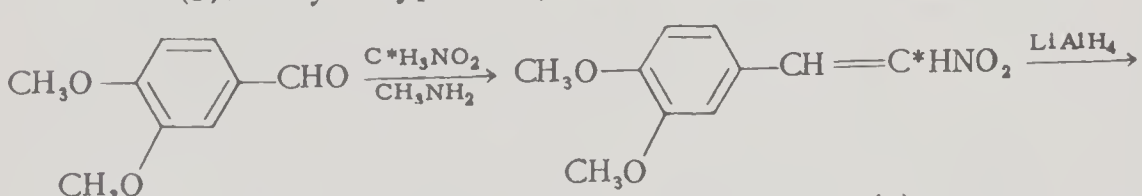
A. Procedure

(a) (p-*Methoxyphenyl)acetonitrile-1-*C^{14}. A solution of 20 g. of freshly prepared *p*-methoxybenzyl chloride in 100 ml. of acetone is mixed with 7 g. of sodium cyanide-C^{14} dissolved in 10 ml. of water (see phenylacetonitrile-1-C^{14}). The mixture is stirred and refluxed for 9 hours, then allowed to stand for 3 days. Water (300 ml.) is added, and the mixture is extracted with three 150-ml. portions of ether. The extract is washed with water, dried and distilled; yield 13.9 g. (67%), b.p. 95.5–97.0° (0.7 mm.).

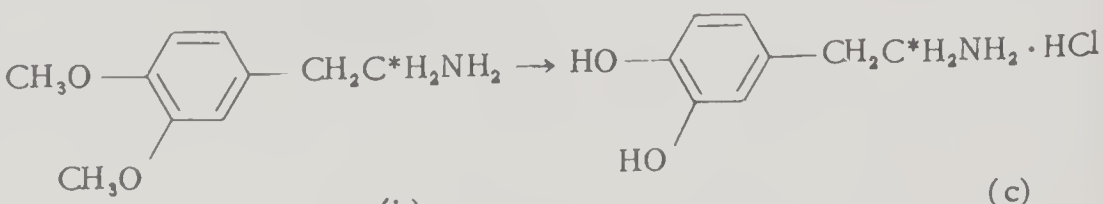
(b) p-*Methoxyphenethylamine-*α-C^{14}. (*p*-Methoxyphenyl)acetonitrile-1-C^{14} (14 g.) is reduced with 9 g. of lithium aluminum hydride according to the general procedure for reduction of nitriles described by Amundsen and Nelson;[1] see propylamine-1-C^{14} perchlorate. The yield is 5 g. (35%), b.p. 78–82° (0.7 mm.), and an additional 8.5% is isolated as the crude hydrochloride from the distillation residue. The hydrochloride melts at 213.5–214.5°, after recrystallization from a mixture of ether and absolute alcohol.

[1] L. H. Amundsen and L. S. Nelson, J. Am. Chem. Soc., 73, 242 (1951).

4-(2-AMINOETHYL-2-C^{14})PYROCATECHOL HYDROCHLORIDE
(3,4-Dihydroxyphenethylamine-α-C^{14} Hydrochloride)



(a)



(b) (c)

G. A. Swan and D. Wright, J. Chem. Soc., *1954*, 381.

A. Procedure

(a) *3,4-Dimethoxy-β-nitrostyrene-β-C^{14}*, [*2-(3,4-Dimethoxyphenyl)-1-nitroethylene-1-C^{14}*](Note 1). To a mixture of 0.25 g. of nitromethane-C^{14} and 0.8 g. (15% excess) of veratraldehyde in 2 ml. of methanol is added a solution of 0.020 g. of methylamine hydrochloride and 0.025 g. of sodium carbonate in 0.25 ml. of methanol. The mixture is stored in the dark for 3 days, then chilled, and the product is collected and washed with methanol; yield 0.7 g. (82%), m.p. 140° (Note 2).

(b) *3,4-Dimethoxyphenethylamine-α-C^{14}*, [*2-(3,4-Dimethoxyphenyl)ethylamine-1-C^{14}*]. To a solution of 0.8 g. of lithium aluminum hydride in 40 ml. of ether is added 0.5 g. of the 3,4-dimethoxy-β-nitrostyrene-β-C^{14} during 20 hours by the Soxhlet technique. To the cooled solution is added water, followed by sodium hydroxide; the aqueous phase is separated and extracted with ether. The combined extract is dried over sodium sulfate, and concentrated, and the residue is distilled; yield 0.32 g. (74%), b.p. 120° (bath temperature) (1 mm.).

(c) *4-(2-Aminoethyl-2-C^{14})pyrocatechol Hydrochloride.* The demethylation is accomplished by heating the ether with about 3 ml. of concentrated hydrochloric acid for 2 hours at 140–160° in a sealed tube. The mixture is evaporated to dryness, and the residue is precipitated from alcohol solution by the addition of ether; yield 0.24 g., m.p. 238°. The yield of product recrystallized from 6 *N* hydrochloric acid is 0.19 g., m.p. 238–239° (Note 3).

B. Notes

1. The procedure is an adaptation of the method of Gairaud[1] for preparing ω-nitrostyrenes.

2. Heating the methylamine-catalyzed reaction mixture reduces the yield by forming a high-melting, less-soluble polymer, which becomes the only product if the mixture is allowed to stand long enough. The nitrostyrenes are purified by recrystallization from methanol or dilute acetic acid.

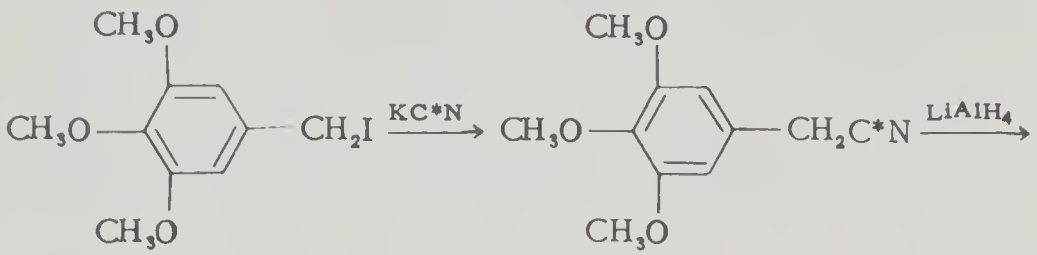
3. Directions are given by Swan for converting these compounds to C^{14}-melanin, with approximately 96% of the carbon dioxide liberated in the process coming from the benzene ring.

C Other Preparations

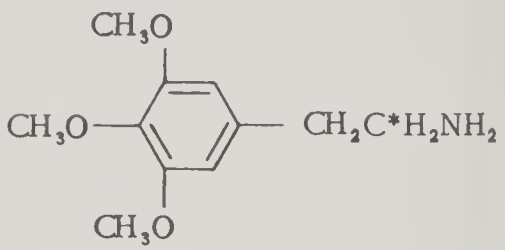
In the same manner from veratraldehyde-C^{14} and nitromethane (15% excess) are prepared: 3,4-dimethoxy-β-nitrostyrene-α-C^{14} (87% yield), 3,4-dimethoxyphenethylamine-β-C^{14} and 4-(2-aminoethyl-1-C^{14})pyrocatechol hydrochloride (Note 3).

[1]C. B. Gairaud and G. R. Lappin, J. Org. Chem., *18*, 1 (1953).

3,4,5-TRIMETHOXYPHENETHYLAMINE-α-C^{14} (C_1^{14}-Mescaline)



(a)



(b)

W. Block and K. Block, Ber., *85*, 1009 (1952).

A. Procedure

(a) *(3,4,5-Trimethoxyphenyl)acetonitrile-1-*C^{14}. To a stirred mixture of 0.213 g. of potassium cyanide-C^{14}, 0.25 ml. of carbon dioxide-free water and 2 ml. of alcohol, heated at 90° under reflux, is added 0.033 g. of 3,4,5-trimethoxybenzyl iodide (Note 1) every minute for 30 minutes, an additional 0.5 ml. of water being added at 10 minutes. The mixture is heated for 15 minutes, then cooled, treated with 5 ml. of benzene and evaporated under reduced pressure at 40°. The residue is treated with benzene and the potassium iodide is separated by filtration and washed with benzene. The filtrate is washed with water and evaporated to dryness. Purification is effected by triturating the residue with 5 ml. of cyclohexane for 30 minutes (Note 2), then decanting the liquor and melting the solidified product at 80° under vacuum to remove the last traces of solvent.

(b) *3,4,5-Trimethoxyphenethylamine-α-*C^{14}. To a stirred solution of the C^{14}-nitrile in 15 ml. of absolute ether at 0° is added dropwise a solution of 0.250 g. of lithium aluminum hydride in 25 ml. of ether. The excess hydride is decomposed, within 5–10 minutes after the addition, by slow addition of 0.4 ml. of water. An additional 3 ml. of water and 0.4 ml. of of 2 *N* sodium hydroxide are added, and the mixture is thoroughly stirred at 30°. The ether solution is decanted, the aluminum hydroxide is washed with several portions of benzene, and the combined filtrate is evaporated under vacuum. The residue is distributed between 5 ml. of ether and 5 ml. of water in a separatory funnel, and the aqueous phase is titrated to pH 6 with 0.1 *N* sulfuric acid and use of a glass electrode.

The aqueous phase is separated and washed with two portions of ether, the extract being resubmitted to the reduction step (Note 3). The combined aqueous solutions are washed with three 5-ml. portions of chloroform and one portion of cyclohexane, then evaporated to dryness under vacuum (Note 4). The salt is triturated with 1 ml. of absolute alcohol, then collected on a filter and washed with 1 ml. of absolute alcohol. The free base is liberated from the residue with aqueous sodium hydroxide and extracted into chloroform solution. Titration with sulfuric acid, as before, and evaporation of the aqueous phase give 0.180 g. of product (Note 5), m.p. 183–186°; yield 20% based on potassium cyanide. The molar specific activity is 63% of that of the starting material (Note 6).

B. Notes

1. Directions are given by Block for chlorinating 3,4,5-trimethoxybenzyl alcohol with anhydrous hydrogen chloride, then exchanging the halogen by treatment with sodium iodide in acetone, giving 3,4,5-trimethoxybenzyl iodide, m.p. 57–58° (dec.).

2. Trial experiments have shown that further purification of the nitrile is indispensable for the success of the reduction step. Vacuum distillation is undesirable with so little material.

3. The yield is raised appreciably by evaporating the extract to dryness, then dissolving the residue in 15 ml. of absolute ether and repeating the reduction step twice, using 80% and 60% of the specified quantities of reagents.

4. Purification of the amine through formation of the picrate (m.p. 217–218°) results in lower recovery.

5. $C_{11}H_{17}O_3N \cdot 1/2\ H_2SO_4 \cdot H_2O$.

6. The loss of specific activity is believed to occur during the reduction.

N,*N*-DIMETHYL-2-DIPHENYLMETHOXY-C^{14}-ETHYLAMINE HYDROCHLORIDE (C_1^{14}-Benadryl)

METHOD I

$$(C_6H_5)_2C^*O \xrightarrow[Zn]{NaOH} (C_6H_5)_2C^*HOH \xrightarrow{Na} (C_6H_5)_2C^*HONa \xrightarrow{ClCH_2CH_2N(CH_3)_2}$$

(a)

$$(C_6H_5)_2C^*HOCH_2CH_2N(CH_3)_2 \xrightarrow{HCl} (C_6H_5)_2C^*HOCH_2CH_2N(CH_3)_2 \cdot HCl$$

(b)

T. A. Geissman, Atomic Energy Commission Report, UCRL-1233; Nuc. Sci. Abstracts, 5, 4722 (1951).

A. Procedure

(a) *Benzhydrol-α-C*^{14}, (*Diphenylmethanol-C*^{14}) (Note 1). To a refluxing solution of 2.38 g. of benzophenone-C^{14}, 2.5 g. of potassium hydroxide and 25 ml. of ethanol is added 2.5 g. of zinc dust in one portion. Following the momentary vigorous reaction, the mixture is refluxed for 1 hour, then filtered onto ice (Note 2). The filtrate is treated with 15 ml. of 6 *N* hydrochloric acid and stored overnight in ice. The crystalline product is collected, washed and dried; yield 2.01 g., 84% (Note 3).

(b) N,N-*Dimethyl-2-diphenylmethoxy-C*^{14}*-ethylamine Hydrochloride.* To a solution of 2.01 g. of benzhydrol-α-C^{14} in 40 ml. of dry xylene is added 0.5 g. of sodium. The mixture is refluxed under nitrogen for 6 hours, then treated with 4.0 g. of freshly distilled 2-chloro-*N*,*N*-dimethylethylamine, and heating is continued for 4 hours. The mixture is filtered onto ice, the salt is washed with ether, and the combined ether-xylene solution is extracted with a total of 25 ml. of 2 *N* hydrochloric acid. The extract is washed with ether, then made alkaline and extracted with ether. The extract is dried over potassium carbonate and evaporated, yielding 2.25 g. of crude product (81%). The yellow oil is distilled under vacuum, and the distillate, dissolved in 5 ml. of dry ether, is treated with 3 ml. of 4 *N* ethanolic hydrogen chloride, followed by an excess of dry ether. The mixture is seeded and cooled overnight to crystallize the oily hydrochloride; yield 2.27 g. Purification is accomplished by dissolution in 5 ml. of hot isopropyl alcohol, filtration through a bed of carbon, washing with 5 ml. of the solvent and dilution of the filtrate with 20 ml. of dry ethyl acetate and 10 ml. of absolute ether. The yield of crystalline product is 1.59 g. (Note 4), and paper chromatography and radioautography indicate that the product is homogeneous (Note 5).

METHOD II

$$(C_6H_5)_2C^*O \xrightarrow{Al(OC_3H_7\text{-}i)_3} (C_6H_5)_2C^*HOH \xrightarrow{CH_3COBr}$$

$$(C_6H_5)_2C^*HBr \xrightarrow{HO(CH_2)_2N(CH_3)_2} (C_6H_5)_2C^*HOCH_2CH_2N(CH_3)_2 \cdot HCl$$

R. W. Fleming and G. Rieveschl, Jr., Abstracts, 112th Meeting, Am. Chem. Soc., 1947, 16K.

A. Procedure

Benzophenone-C^{14} is reduced to benzhydrol-α-C^{14} in 99% yield by the Meerwein-Ponndorf-Verley method.[1] The carbinol is halogenated by treatment with acetyl bromide in benzene solution, and the bromodiphenylmethane-C^{14} is condensed with 2-dimethylaminoethanol in xylene solution, forming the product in 70% yield (55% based on barium carbonate).

B. Notes

1. The procedure is adapted from that of Wiselogle and Sonneborn.[2]

2. The filtered zinc dust is pyrophoric if dried in contact with combustibles.

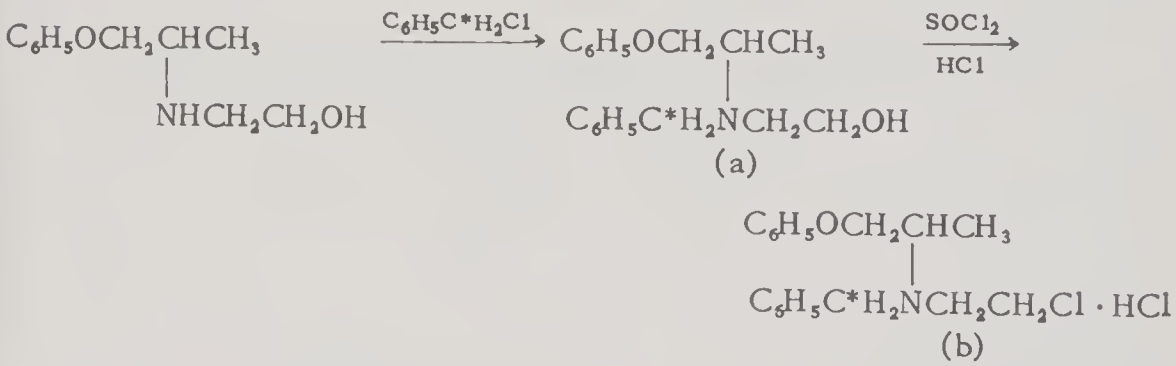
3. In trial runs the yields were nearly quantitative.

4. The combined mother liquors are extracted with dilute hydrochloric acid, and the recovered benadryl is converted to the methiodide by treatment of the free base with methyl iodide in ether solution. The 2-(diphenylmethoxy-C^{14})ethyltrimethylammonium iodide is recrystallized from absolute alcohol; yield 0.48 g.

5. The one-dimensional paper chromatogram is developed on Whatman #1 paper in methanol (10 ml.)-hydrochloric acid (1 drop, 6 *N*); R_f 0.88.

[1]*Organic Reactions*, Vol. II, Wiley, New New York, 1944, p. 203.
[2]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 90.

N-(2-CHLOROETHYL)-*N*-(1-METHYL-2-PHENOXYETHYL)BENZYLAMINE-α-C^{14} HYDROCHLORIDE



E. J. Nikawitz, W. S. Gump, J. F. Kerwin and G. E. Ullyot, J. Am. Chem. Soc., 74, 2438 (1952).

A. Procedure

(a) *2-[Benzyl-α-C^{14}-(1-methyl-2-phenoxyethyl)amino]ethanol.* A mixture of 1.09 g. of 2-(1-methyl-2-phenoxyethylamino)ethanol, 0.684 g. of benzyl-α-C^{14} chloride, 0.29 g. of anhydrous sodium carbonate and 7 ml. of absolute alcohol is refluxed for 10 hours. The alcohol is removed in an air stream, and the residue is mixed with small amounts of water and ether. The ether solution is separated, dried, concentrated to small volume and transferred to the first bulb of a three-bulb distilling apparatus (Note 1) with a small amount of ether, which is then removed by heating in a water-bath at 40–50°. The residue is distilled at 0.15 mm. by heating bulbs 1 and 2 in an air-bath at 142° for 15 minutes, thus allowing the forerun to collect in bulb 3. The product is then distilled into bulb 2

by heating bulb 1 in the air-bath at 160–178° for about 25 minutes. Bulb 2 is cut away from the system; yield 1.1205 g. of colorless oil.

(b) N-(*2-Chloroethyl*)-N-(*1-methyl-2-phenoxyethyl*)*benzylamine-α-C*^{14} *Hydrochloride*. A solution of the amino alcohol in 3 ml. of dry chloroform, in a Pyrex tube (Note 2), is cooled with an ice-bath, and dry hydrogen chloride is added until the solution is acidic. Thionyl chloride is added slowly to the cooled solution, which is then heated at 50–60° for two hours.[1] The solvent is removed under reduced pressure, and crystallization of the residue is induced by trituration with ether. The product is recrystallized from alcohol and ether; yield 1.0414 g., m.p. 137.5–140°.

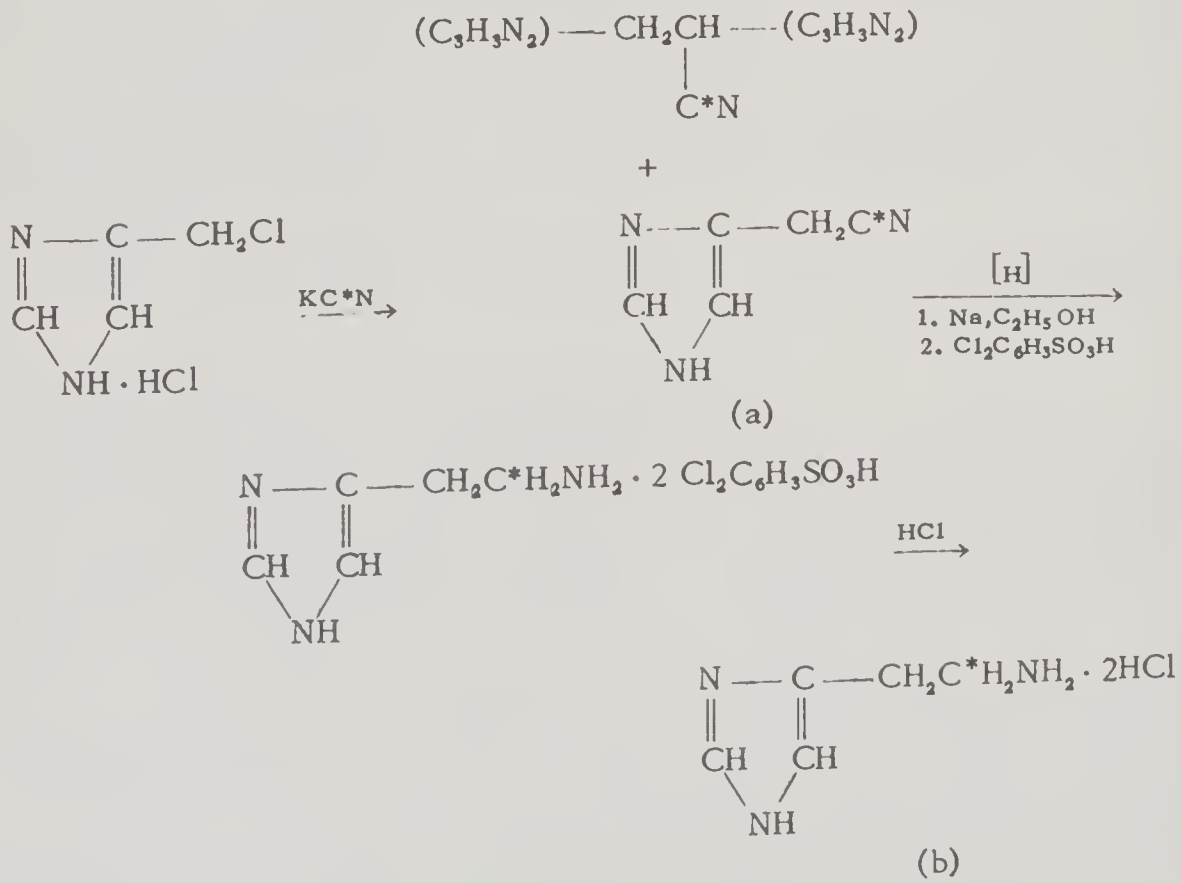
B. Notes

1. A diagram of the apparatus is presented by Nikawitz. Three bulbs of about 37 mm. diameter are connected by 8-mm. tubing. The air-bath is in the form of an oven.

2. The tube is 100 mm. × 22 mm. I.D.

[1] J. F. Kerwin, G. C. Hall, F. L. Milnes, I. H. Witt, R. A. McLean, E. Macko, E. J. Fellows and G. E. Ullyot, J. Am. Chem. Soc., 73, 4162 (1951).

4(or 5)-IMIDAZOLEËTHYLAMINE-α-C^{14} DIHYDROCHLORIDE
(C_1^{14}-Histamine Dihydrochloride)



L. P. Bouthillier and M. Goldner, Arch. Biochem., 44, 251 (1953).

A. Procedure

(a) *4(or 5)-Imidazoleacetonitrile-C^{14}*. 4(or 5)-(Chloromethyl)imidazole is reacted with 1 mmole of potassium cyanide-C^{14} by a suitable adaptation of the method of Pyman,[1] which follows.

To a stirred solution of 90 g. of potassium cyanide in 100 ml. of water, maintained at 0°, is added dropwise a solution of 30 g. of 4(or 5)-(chloromethyl)imidazole hydrochloride (Note 1) in 135 ml. of absolute alcohol during 30 minutes. The combined filtered solution and alcohol washings are mixed with 180 ml. of aqueous 10% sodium carbonate and evaporated to dryness under vacuum. The residue is extracted into warm ethyl acetate solution, and the solvent is distilled. The product is crystallized from hot water; yield 8.5 g. (40%, Note 2), m.p. 138–140° (cor.) (Note 3).

(b) *4(or 5)-Imidazoleëthylamine-α-C^{14} Dihydrochloride*. The imidazoleacetonitrile-C^{14} is reduced by the action of sodium and alcohol, according to the method of Koessler,[2] which follows. The product is isolated as the "bis(*o*-dichlorobenzenesulfonate)" (Note 4) and converted to the dihydrochloride (m.p. 229–231°) by treatment with hydrochloric acid. Paper chromatography of the product in 1-butanol-95% ethanol-concentrated ammonium hydroxide (8:1:3 v/v) gives a single spot with the same R_f value as an authentic specimen.

To a solution of 5 g. of 4(or 5)-imidazoleacetonitrile in 50 ml. of boiling absolute ethanol is added 8 g. (2 equivalents) of sodium in small pieces over 5 minutes. The mixture is refluxed until the metal is dissolved, 20 ml. of alcohol being added at 45 minutes. The cooled mixture is diluted with 50 ml. of water and concentrated to 25 ml. under reduced pressure to remove alcohol and ammonia. The solution is diluted with 25 ml. of water and extracted with five 50-ml. portions of amyl alcohol (Note 5). The combined extract is dried over sodium carbonate, and the filtered solution is extracted with three 100-ml. portions and two 50-ml. portions of 0.5 *N* hydrochloric acid. The combined extract (pink) is heated for 1 hour on a steam-bath, then decolorized with carbon, and the filtrate is evaporated to dryness at 60° under reduced pressure (Note 6); yield 7.2–7.8 g. The residue is triturated with 1 ml. of 20% hydrochloric acid and 10 ml. of absolute ethanol, then digested on a steam-bath and stored overnight in a refrigerator. The product is collected, washed with alcohol and dried for 2 hours at 100° and 48 hours under vacuum; yield 4.2–4.8 g. (50–56%), m.p. 244–246° (cor.) (Note 7).

B. Notes

1. Directions are given by Pyman[1] for preparing the starting material by the action of phosphorus pentachloride on 4(or 5)-imidazolemethanol,[3] or by the action of thionyl chloride on the picrate.[4]

2. The yield may be raised to 50% by the tedious separation, by fractional recrystallization from water, of the 4(or 5)-imidazoleacetonitrile hydrogen oxalate (m.p. 194° cor., dec.) and the α,β-bis[4(or 5)-imidazole]propionitrile-C^{14} hydrogen oxalate (m.p. 181–182° cor., dec.) formed by treating the mother liquor with 9 g. of oxalic acid. The product hydrochloride, crystallized from absolute alcohol, melts at 168–169° (cor.).

3. Bouthillier[1] has reported the conversion of the nitrile, by the method of Pyman,[1] to ethyl 4(or 5)-imidazoleacetate-C^{14}, 4(or 5)-imidazoleacetic-C^{14} acid hydrochloride and 4(or 5)-imidazoleacetic-C^{14} acid monohydrate. The free acid is liberated with silver carbonate, and the monohydrate is crystallized from water-acetone; m.p. 217–218°; yield 18% based on 4(or 5)-(chloromethyl)imidazole hydrochloride at the 6.5-mmole level. The free acid may be sublimed, at 160–170° (< 50 μ). The chromatography of the acid and use of the Pauly reaction for characterizing imidazoles are described; also see 4(or 5)-imidazolepropionic-α-C^{14} acid, Note 4.

4. This compound might be presumed to be the bis(3,4-dichlorobenzenesulfonate).

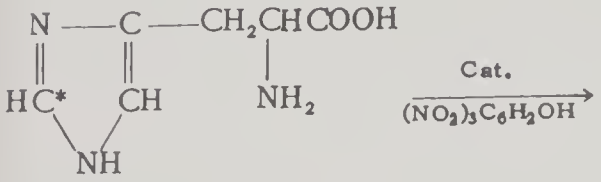
5. The requisite complete phase separation may be quickly achieved by centrifuging. The strongly alkaline solution may be worked up for recovery of 4(or 5)-imidazoleacetic acid hydrochloride, m.p. 226–228° (cor.).

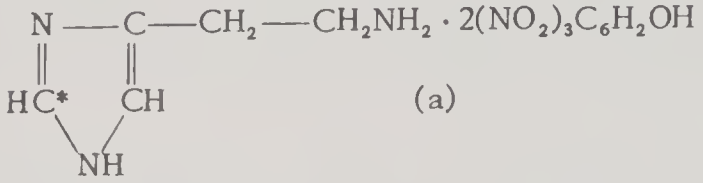
6. Any sodium chloride deposited upon reaching a small volume (15 ml.) is filtered off, after diluting the mixture with 30 ml. of absolute alcohol.

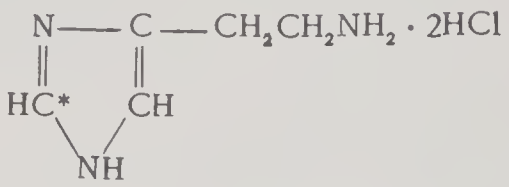
7. The dipicrate from water melts at 238–240°. Analysis shows the the product to be 94.8% pure histamine dihydrochloride and 3.92% sodium chloride.

[1]F. L. Pyman, J. Chem. Soc., 99, 668 (1911).
[2]K. K. Koessler and M. T. Hanke, J. Am. Chem. Soc., *40*, 1716 (1918).
[3]*Organic Syntheses*, Vol. 24, Wiley, New York, 1944, p. 64.
[4]A. H. Mehler, H. Tabor and H. Bauer, J. Biol. Chem., *197*, 475 (1952).
[5]L. P. Bouthillier and G. Leveille, J. Am. Chem. Soc., *75*, 4075 (1953).

4(or 5)-IMIDAZOLEËTHYLAMINE-2-C^{14} DIHYDROCHLORIDE (C_1^{14}-Histamine Dihydrochloride)







(b)

R. W. Schayer, J. Am. Chem. Soc., *74*, 2440 (1952); J. Biol. Chem., *196*, 469 (1952).

A. Procedure

(a) *4(or 5)-Imidazoleëthylamine-2-C^{14} Dipicrate.* A mixture of histidine decarboxylase (Note 1) and 0.0184 g. of L-histidine-2-C^{14} is incubated in a Warburg flask at 30° until carbon dioxide evolution is complete (65 min.). The mixture is made strongly alkaline and extracted with six portions of amyl alcohol. The solvent is evaporated under vacuum, and to a solution of the residue in 3 ml. of water is added a hot solution of 0.060 g. of picric acid in 4 ml. of water. The hot filtered solution is allowed to crystallize; yield 0.0443 g. (65%), m.p. 238–242° (dec.) (Note 2). Additional product is available by recrystallizing carrier from the mother liquor; the picrate may be recrystallized from water.

(b) *4(or 5)-Imidazoleëthylamine-2-C^{14} Dihydrochloride.* A solution of the 4(or 5)-imidazoleëthylamine-2-C^{14} dipicrate in 0.15 *N* hydrochloric acid is extracted with ether, and the aqueous solution is evaporated to dryness. Paper chromatography shows the product to be free from radioactive contamination (Note 3).

B. Notes

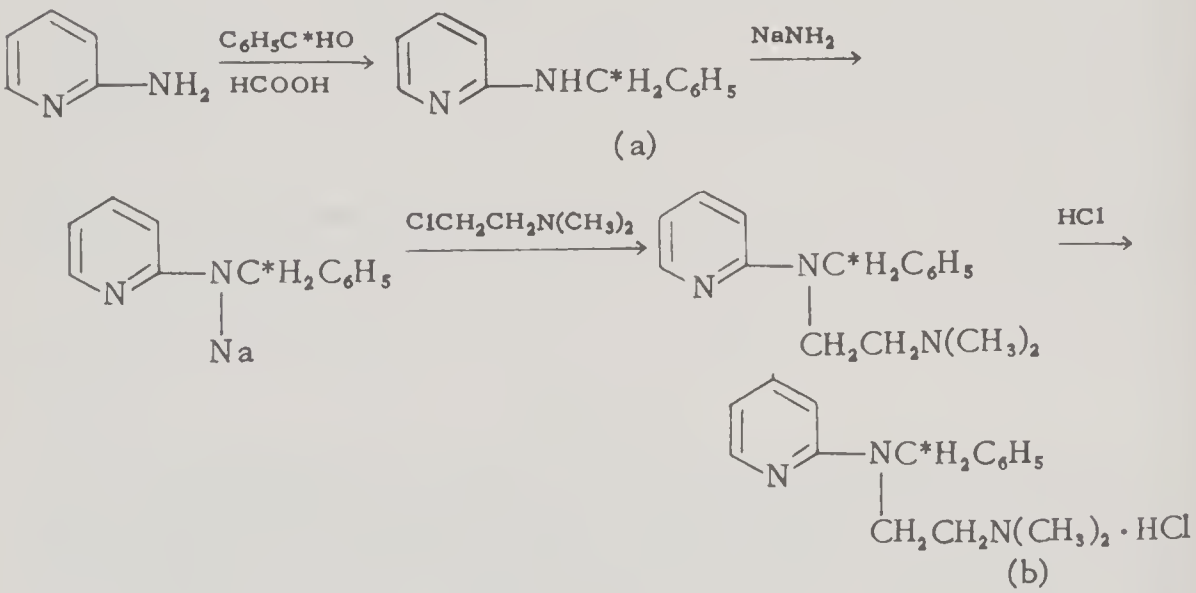
1. The enzyme is prepared by incubating 0.050 g. of an acetone powder preparation of *Lactobacillus* with 5 ml. of McIlvaine buffer at pH 4.8 for 6 hours. The cells are removed by centrifugation, and the liquor is used.

2. Histamine has been prepared by Ewins and Pyman,[1] in moderate yields, by the action of hydrochloric acid upon histidine at 270°.

3. A single sharp radioactive peak (R_f 0.80) is obtained by chromatography in butanol-ammonia; L-histidine-2-C^{14}, R_f 0.15. The product possesses the same pharmacological activity as an authentic sample.

[1]A. J. Ewins and F. L. Pyman, J. Chem. Soc., *1911*, 339.

2-[BENZYL-α-C^{14}-(2-DIMETHYLAMINOETHYL)AMINO]PYRIDINE HYDROCHLORIDE (C_1^{14}-Pyribenzamine Hydrochloride)



T. A. Geissman, Atomic Energy Commission Report, UCRL-1253; Nuc. Sci. Abstracts, 5, 4722 (1951).

A. Procedure (Note 1)

(a) *2-(Benzylamino-α-C^{14})pyridine* (Note 2). The crude benzaldehyde-C^{14} prepared from 13.1 mmoles of benzoic-C^{14} acid (Note 3) is mixed with 2.0 g. of 2-aminopyridine and 2 ml. of 98% formic acid, and the mixture is heated under reflux at 135° for 6 hours. The mixture is treated with water and 10 ml. of 6 *N* sodium hydroxide, then extracted with ether

(Note 4) which, in turn, is extracted with *N* hydrochloric acid. The acid solution is basified and cooled to crystallize the product. The residual ether solution, smelling strongly of benzaldehyde, is dried and concentrated, and the oily residue (<1 g.) is put through the synthesis with one-half quantities of the reagents. The combined crude crystalline product (0.972 g., 41%) is mixed with 1.00 g. of pure carrier and recrystallized from dilute alcohol; yield 1.93 g. (Note 5).

(b) *2-[Benzyl-α-C^{14}-(2-dimethylaminoethyl)amino]pyridine Hydrochloride.* To 1.00 g. of sodamide is added a solution of 1.93 g. of 2-(benzylamino-α-C^{14})pyridine in 20 ml. of benzene, and the stirred mixture is refluxed for 3.5 hours. To the solution is added 2.5 ml. of distilled (2-chloroethyl)dimethylamine in 5 ml. of benzene, and the mixture is refluxed for 7 hours, then filtered into ice and water. The benzene extract is washed with water, then passed through a column of anhydrous potassium carbonate and concentrated. The residual oil is distilled at 210–220° (15 mm.); yield 2.07 g. (77%). To a solution of the 2-[benzyl-α-C^{14}-(2-dimethylaminoethyl)amino]pyridine in 10 ml. of dry ethyl acetate is added 1.9 ml. of 4.1 *N* ethanolic hydrogen chloride (Note 6), followed by absolute ether to incipient turbidity; then the mixture is seeded and stored overnight at 0°. The product is collected, dissolved in 8 ml. of hot isopropyl alcohol and decolorized with carbon, the residue being washed with 1 ml. of the hot alcohol and 5 ml. of hot ethyl acetate. The cooled filtrate is diluted with 15 ml. of absolute ether, and the precipitated product (1.57 g.) is recrystallized from absolute ethanol-ether; yield 1.282 g., m.p. 185–186° (Note 7).

B. Notes

1. The method is essentially that described by Biel[1] for preparing substituted ethylenediamines; also see Clark.[2] Five alternative nonisotopic preparative methods are described by Huttrer.[3]

2. The procedure is adapted from that of Tschitschibabin and Knunjanz, yield 90%.[4] A survey of the Leuckart reaction is presented by Moore.[5]

3. Although the crude benzaldehyde-C^{14} (2.6 g.), prepared by the method of McFadyen and Stevens, weighs nearly twice the theoretical amount, it is used without further purification.

4. The alkaline solution contains approximately 9% of the original radioactivity.

5. 2-Benzylaminopyridine distills at 137–144° (0.02 mm.), m.p. 95–96° (cor.).[3]

6. The amount of hydrogen chloride is based on the weight of distillate. The dihydrochloride is oily.

7. The combined mother liquors are extracted with dilute hydrochloric acid, and the recovered product is converted to the dipicrate, m.p. 182–183° from acetic acid. The picrate is decomposed with aqueous ethanolamine, and the free base is treated with an ethereal solution of methyl iodide. The yield of the methiodide, recrystallized from methanol-ether, is 0.44 g., m.p. 169–170° (dec.). The molar specific activities of all compounds are in agreement with the expected values. Pyribenzamine boils at 138–142° (0.01 mm.), and the hydrochloride melts at 193° (cor.).[3]

[1]J. H. Biel, J. Am. Chem. Soc., *71*, 1306 (1949).

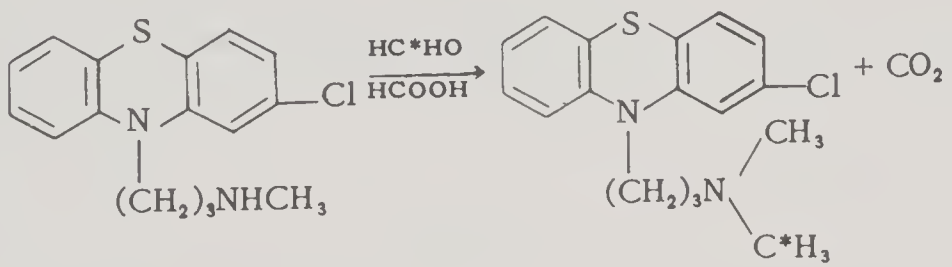
[2]J. H. Clark, R. C. Clapp, J. R. Vaughan, Jr., L. H. Sutherland, R. Winterbottom, G. W. Anderson, J. D. Forsythe, J. Blodinger, S. L. Eberlin and J. P. English, J. Org. Chem., *14*, 216 (1949).

[3]C. P. Huttrer, C. Djerassi, W. L. Beears, R. L. Mayer and C. R. Scholz, J. Am. Chem. Soc., 68, 1999 (1946).

[4]A. E. Tschitschibabin and I. L. Knunjanz, Ber., 64, 2839 (1931).

[5]*Organic Reactions*, Vol. V, Wiley, New York, 1949, Chapt. 7.

2-CHLORO-10-(3-DIMETHYLAMINO-C_1^{14}-PROPYL)PHENOTHIAZINE HYDROCHLORIDE (C_1^{14}-Chlorpromazine)



D. G. Ott, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, unpublished work.

A. Procedure (Note 1)

To a solution of 2 ml. of methanol and the formaldehyde-C^{14} prepared from 1.21 mmoles of barium carbonate-C^{14} by Method I is added a solution of 0.500 g. (1.47 mmoles) of 2-chloro-10-(3-methylaminopropyl)phenothiazine hydrochloride (Note 2), 3 ml. of 0.5 *N* sodium hydroxide and 0.35 ml. (9.0 mmoles) of formic acid (Note 3). The solution is heated under reflux for 3 hours on a water-bath at 60–70°, then treated with a few drops of methanol containing 0.025 ml. (0.34 mmole) of 40% formaldehyde solution and heated for another 2 hours, the process being repeated twice. The mixture is transferred with water to a 40-ml. centrifuge cone (Note 4),

made strongly alkaline with 40% sodium hydroxide and extracted 20 times with 1-ml. portions of benzene (Note 5). The extract is washed with water and extracted 20 times with 1-ml. portions of concentrated (36%) hydrochloric acid. The extract is diluted with water to 25% hydrochloric acid (Note 6) and extracted 20 times with 1-ml. portions of chloroform. The extract is concentrated to 3 ml. (Note 7) and diluted with 30 ml. of absolute ether. The product is collected by centrifugation, triturated with ether, separated and dried under vacuum; yield 0.396 g. (76%), radiochemical yield 32% (Note 8).

B. Notes

1. The procedure is an adaptation of the Eschweiler-Clarke modification of the Leuckart reaction for reductive alkylation of amines.[1] See ethyl 1-methyl-C^{14}-4-phenylisonipecotate.

2. The starting material may be prepared from 2-chloro-10-(3-chloropropyl)phenothiazine[2] and methylamine.

3. The amount of formic acid used is that required by the reaction plus sufficient to neutralize the base formed from the reduction of carbon dioxide by lithium aluminum hydride (see formaldehyde-C^{14}).

4. Although the dry salt and the free base are quite stable, aqueous chlorpromazine hydrochloride solutions are sensitive to light and oxygen.

5. The extractive separation is accomplished with a dropping pipette and centrifuge cones.

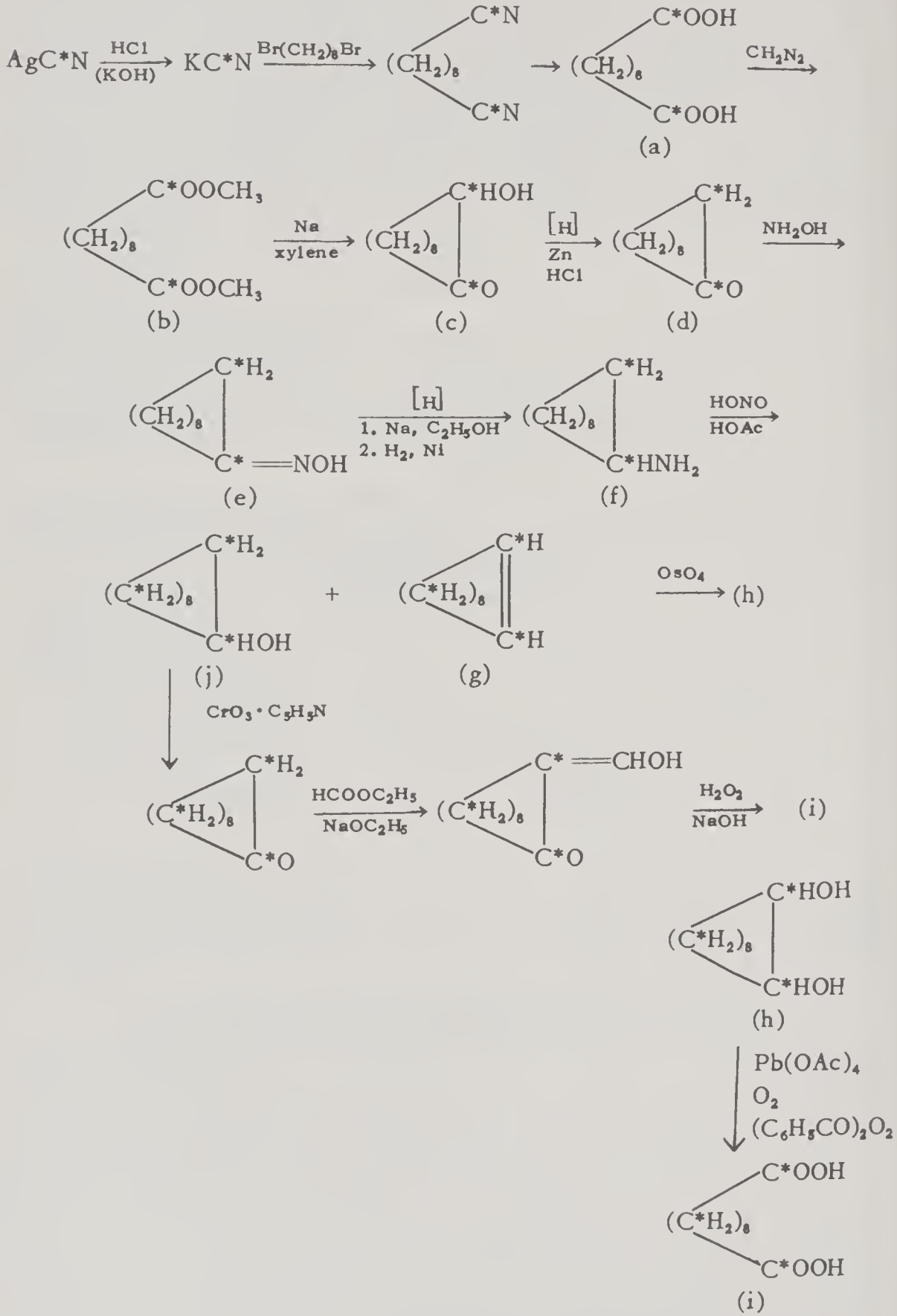
6. This acid concentration is approximately optimum for extraction of the product hydrochloride into chloroform.[3]

7. A glass ebullator tube is used to prevent bumping during the azeotropic drying and concentration on the steam-bath.

8. In trial preparations the over-all chemical and radiochemical yields were 90–98% and 45–58%, respectively. Chlorpromazine hydrochloride melts at 193–194°. The ultraviolet and infrared spectra are identical with those of authentic material.

[1] *Organic Reactions*, Vol. V, Wiley, New York, 1949, p. 301.
[2] R. Charpentier, U.S. 2,645,640; Chem. Abstracts, 49, 3269 (1955).
[3] P. N. Craig, Smith, Kline and French Laboratories, Philadelphia, Pa., private communication.

CYCLODECYLAMINE-1,2-C_2^{14}



V. Prelog, H. J. Urech, A. A. Bothner-By and J. Würsch, Helv. Chim. Acta, 38, 1095 (1955).

A. Procedure

(a) *Decanedioic-1,10-C_2^{14} Acid, (Sebacic-C_2^{14} Acid).* To 0.2680 g. (2.0 mmoles) of silver cyanide-C^{14} in a centrifuge cone is added 2.01 ml. of 0.1 *N* hydrochloric acid. The liberated hydrogen cyanide-C^{14} is transferred with a stream of carbon dioxide-free nitrogen, during 3 hours, into a solution of 0.079 g. of potassium in 1 ml. of ethanol, contained in a reaction tube cooled at −78°. The entry tube is cut off and left in the tube, and to the solution of potassium cyanide-C^{14} is added 0.272 g. of 1,8-dibromoöctane and 0.020 g. of potassium iodide. The mixture is refluxed for 20 hours, then cooled, mixed with a solution of 0.4 g. of potassium hydroxide in 1 ml. of alcohol and 1.5 ml. of water, and refluxed for an additional 20 hours to hydrolyze the decanedinitrile-1,10-C_2^{14}. The mixture is diluted with water, washed with three 20-ml. portions of ether, then acidified with sulfuric acid and extracted with ether. The extract is washed with water, dried over sodium sulfate and evaporated; yield 0.198 g. (78% based on barium carbonate-C^{14}), m.p. 124.5–129.5° (Note 1).

(b) *Methyl Decanedioate-1,10-C_2^{14}.* The acid (0.305 g.) is esterified in absolute ether solution by the addition of diazomethane in ether. Evaporation under reduced pressure, dilution with 2.5 g. of carrier and vacuum distillation yield 2.78 g. of product, b.p. 108–112° (40 μ). The still is scavanged with 0.97 g. of carrier, raising the yield to 3.7 g. of product, which readily crystallizes (Note 2).

(c) *2-Hydroxycyclodecanone-1,2-C_2^{14}.* The product is prepared from 3.7 g. of the ester, 300 ml. of xylene and 1.7 g. of sodium according to the procedure of Prelog,[1] which follows; yield 1.287 g. (47%), b.p. 118–131° (0.1 mm.).

To a stirred mixture of 170 g. of sodium in 4700 ml. of anhydrous xylene at 130° under nitrogen in a specially designed steel apparatus is added 410 g. of methyl sebacate in 200 ml. of xylene during 28 hours. The mixture is stirred for 2 hours, treated with 200 ml. of methanol and acidified with 40% sulfuric acid. The xylene extract is washed with sodium carbonate and water, and fractionally distilled; yield 53%, b.p. 70–88° (0.1 mm.). The product soon crystallizes (Note 3).

(d) *Cyclodecanone-1,2-C_2^{14}* (Note 4). The acyloin (1.287 g.) is reduced with 3.27 g. of zinc wool and 12 ml. of purest concentrated hydrochloric acid in 10 ml. of glacial acetic acid; yield 0.969 g. (83%), b.p. 98–104° (10 mm.).

(e) *Cyclodecanone-1,2-C_2^{14} Oxime.* A mixture of 1.834 g. of cyclodecanone-1,2-C_2^{14}, 20 ml. of alcohol, 1.86 g. of pyridine and 1.6 g. of hydroxylamine hydrochloride is shaken until solution takes place. The alcohol is removed under vacuum, and the residue is stirred with 15 ml. of water. The product is collected, washed with ice-cold dilute hydrochloric acid and water, and recrystallized from 90% alcohol; yield 1.706 g. (85%), m.p. 79.5–80.5°.

(f) *Cyclodecylamine-1,2-C_2^{14} Hydrochloride* (Note 5). The reduction of 1.702 g. of the oxime is carried out in 60 ml. of absolute alcohol with 3.7 g. of sodium. The frozen reaction mixture is acidified with cold dilute hydrochloric acid, and the alcohol is distilled under vacuum. The solution is extracted with pentane to remove nonbasic material (Note 6), then is basified with 40% potassium hydroxide and continuously extracted with ether. The extract is dried over potassium hydroxide and distilled, yielding 0.556 g. of cyclodecylamine-1,2-C_2^{14}, b.p. 117–121° (11 mm.). The distillation apparatus is washed with dilute hydrochloric acid (Note 7), and the wash liquor is basified and steam-distilled. The distillate is titrated with *N* hydrochloric acid (2.25 ml.) and lyophilized. The residue is recrystallized from ethyl acetate; yield 0.312 g., m.p. 179.5–181°.

The nonbasic by-product (0.580 g.) is added to 0.5 g. of chromium trioxide in 5–8 ml. of anhydrous pyridine (Note 8). The mixture is stored at room temperature for 15 hours, decomposed with 15 ml. of ice water and extracted with pentane. The extract is washed with water, dilute hydrochloric acid and water, then dried over sodium sulfate and evaporated through a Vigreux column. The residual cyclodecanone-1,2-C_2^{14} is converted to the oxime (yield 0.536 g., m.p. 79–80.5°), which is hydrogenated for 15 hours over Raney nickel at 100–110 atmospheres in 20 ml. of alcohol at 100°. The filtered solution is acidified with hydrochloric acid and lyophilized, and the residue is recrystallized from ethyl acetate; yield 0.363 g., m.p. 180–181°. The total yield is 70.4%.

Cyclodecylamine-1,2-C_2^{14} is obtained by basifying a concentrated aqueous solution of the hydrochloride with saturated potassium hydroxide, extracting with ether and evaporating under vacuum (Note 9).

(g) C_2^{14}-cis-*Cyclodecene* (Note 10). To a solution of 12.5 mmoles of cyclodecylamine-1,2-C_2^{14} in 3.75 g. of glacial acetic acid and 40 ml. of water is added a saturated aqueous solution containing 3.45 g. of sodium nitrite. After standing overnight, the mixture is refluxed for 1 hour with 30 ml. of 30% potassium hydroxide solution and extracted with pentane. The extract is washed with dilute hydrochloric acid, dilute sodium hydroxide and water, then dried over sodium sulfate and evaporated through a Vigreux column, yielding 1.866 g. of neutral, yellowish oil. The combined oil (Note 11) is separated by chromatography on 65 g. of neutral aluminum oxide (activity III to IV):

Fraction	*Eluent*	*Eluate (g.)*	
1–4	pentane	1.418	colorless oil
5	"	0.026	" "
6–8	"	0.071	" "
9–10	pentane-benzene (9 : 1)	0.028	brown resin
11–12	ether	0.327	colorless, partly crystalline oil
13–18	"	0.035	resin
19–20	ether-methanol (9 : 1)	0.007	"

Fraction 1–4 is distilled at the pressure of a water pump; yield 1.128 g. of C_2^{14}-*cis*-cyclodecene as a colorless oil. The purity is 87.5% (Note 12), making the yield 57% (Note 13). Degradation studies indicate that the isotope is distributed throughout the ring as a result of rearrangement (Note 14).

(h) C_2^{14}-cis-*1,2-Cyclodecanediol* (Note 14). A mixture of 1.065 g. of the crude olefin, 2 g. of osmium tetroxide and a drop of dry pyridine in absolute ether is stored at room temperature for 15 minutes, then shaken for 1 hour with 5.5 g. of potassium hydroxide, 5.5 g. of mannitol, 100 ml. of water and 300 ml. of methylene chloride. The organic layer is washed with dilute hydrochloric acid and water, dried over sodium sulfate and evaporated. The largely-crystalline residue (1.078 g.) is purified by vacuum sublimation and recrystallization from alcohol-benzene; yield 0.831 g. (72%), m.p. 139–140°.

(i) C_2^{14}-*Decanedioic Acid* (Note 14). To a suspension of 1.68 g. of freshly prepared lead tetraacetate and a crystal of benzoyl peroxide in 80 ml. of absolute benzene, through which is passed a stream of dry oxygen under anhydrous conditions, is added a lukewarm solution of 0.483 g. of the diol in 700 ml. of benzene during 2 hours. The stirring and flow of oxygen are continued for 21 hours. The mixture is hydrolyzed with 10 ml. of water and evaporated under reduced pressure. The residue is stirred with dilute hydrochloric acid, and the mixture is extracted with ether, which in turn is extracted with dilute sodium hydroxide (Note 15). Acidification gives the product; yield 0.446 g. (78.5%), m.p. 132.5–133.5° (Note 16).

(j) C_2^{14}-*Cyclodecanol* (Note 10). Fraction 11–12, from the chromatography of C_2^{14}-*cis*-cyclodecene, is converted to C_2^{14}-cyclodecyl *p*-nitrobenzoate by the action of *p*-nitrobenzoyl chloride in pyridine. The ester is recrystallized twice from chloroform-ethyl acetate; yield 0.195 g. (5.1%), m.p. 115–116°.

Fraction 5 is combined with the mother liquors from 1–4 and 6–8, and the mixture is saponified by refluxing for 2 hours with 10 ml. of 20% methanolic potassium hydroxide and evaporated. The residue is extracted with pentane, and the solution is concentrated and converted to the *p*-nitrobenzoate ester; yield 0.395 g. (10.4%), m.p. 115.5–116.5°, making the total yield of ester 15.5%.

The saponification of 0.590 g. of C_2^{14}-cyclodecyl *p*-nitrobenzoate by refluxing for 2 hours with 10 ml. of 20% methanolic potassium hydroxide yields 0.271 g. (90%) of C_2^{14}-cyclodecanol, m.p. 40°. Degradation studies indicate that the isotope is distributed in the ring (Note 17).

B. Notes

1. Pure sebacic acid melts at 133–134.5°, b.p. 295° (100 mm.).
2. Methyl sebacate, m.p. 26.4°, b.p. 175° (20 mm.), n_D^{28} 1.43549.

3. Prelog[2] has given the b.p. 75–85° (0.1 mm.), m.p. 42°.

4. The method is that of Prelog.[2] Cyclodecanone semicarbazone, m.p. 203–204° from methanol, may be decomposed by mixing with phthalic anhydride and steam distilling.

5. The procedure is that of Prelog.[3]

6. The residue (0.580 g.), left by distillation of the solvent through a Vigreux column, is chiefly cyclodecanone-1,2-C_2^{14}.

7. Part of the product becomes bound as the carbonate, despite the fact that carbon dioxide is excluded from nearly all operations.

8. The chromium trioxide-pyridine complex is a useful oxidizing agent[4] for converting hydroxyl groups (associated with an acid-sensitive group) to ketones or aldehydes in yields approaching the theoretical. If the pyridine (20°) is added to the chromic anhydride, the mixture usually inflames; see Poos.[4]

9. Degradation studies, see below, indicate that there is no isotopic rearrangement.

10. This preparation is incidental to a tracer study of the nonclassical rearrangement of cycloamines (C-8 to 11) in the presence of nitrous acid.

11. The unreacted free base (0.121 g.) recovered from the acid extract is subjected again to the action of nitrous acid, yielding 0.112 g. of neutral product.

12. The purity is determined on the basis of uptake in a microhydrogenation in acetic acid over platinum oxide.

13. Fraction 6–8 yields 0.045 g. (1.7%) of C_2^{14}-cyclodecanone semicarbazone, m.p. 199–200°.

14. Radioactive assay of the various degradation products indicates that the isotopic distribution is 59.1% at C–1,10; 24.8% at C–2,9; 3.7% at C–3,8; and 13.1% at C–4,5,6,7, in terms of decanedioic acid, (i). A classical elimination reaction would have given 75% at C–1,10 and 25% at C–2,9.

15. A neutral, oily by-product is left, 0.035 g.

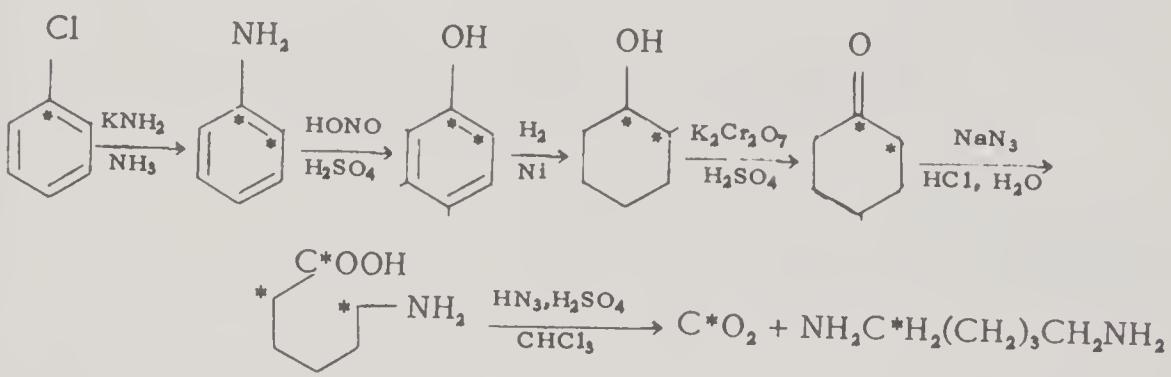
16. Directions are given for converting the acid by the Schmidt reaction to C^{14}-1,8-octanediamine hydrochloride (yield 90.3%), which was assayed as C^{14}-*N,N'*-octamethylenebis(benzamide), m.p. 172–173° from methanol The free base is oxidized with permanganate to C^{14}-octanedioic acid, m.p. 140.5–141.5°. This acid is degraded to C^{14}-1,6-hexanediamine by another Schmidt reaction, then to C^{14}-hexanedioic acid and C^{14}-succinic acid by repetition of the above processes.

17. Directions are given for oxidizing the carbinol with the chromium trioxide-pyridine complex (Note 8), forming C_2^{14}-cyclodecanone, which was isolated as the semicarbazone; yield 85%, m.p. 199.5–201°. The free ketone was obtained by mixing the semicarbazone to a paste with phthalic anhydride and water, and steam distilling. Reaction of the ketone with ethyl formate in the presence of sodium ethoxide forms C_2^{14}-2-(hydroxy-

methylene)cyclodecanone, which is oxidized with alkaline hydrogen peroxide to C_2^{14}-decanedioic acid, m.p. 131–133°. Further degradation is carried out as already indicated. Radioactive assay data indicates that the isotope distribution is: 49.6% at C-1,10; 23.3% at C-2,9; and 24.0% at C-3,4,5,6,7,8, in terms of decanedioic acid, (i). A classical substitution reaction would have given 75% at C-1,10 and 25% at C-2,9.

[1]V. Prelog, K. Schenker and H. H. Günthard, Helv. Chim. Acta, *35*, 1598 (1952).
[2]V. Prelog, L. Frenkiel, M. Kobelt and P. Barman, Helv. Chim. Acta, *30*, 1741 (1947).
[3]V. Prelog, M. Fausy, El Neweihy and O. Häfliger, Helv. Chim. Acta, *33*, 265 (1950).
[4]G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, J. Am. Chem. Soc., *75*, 422 (1953).

ANILINE-1-2-$C^{14}_{1/2}$



J. D. Roberts, H. E. Simmons, Jr., L. A. Carlsmith and C. W. Vaughan, J. Am. Chem. Soc., *75*, 3290 (1953).

A. Procedure

1-Chlorobenzene-1-C^{14} is aminated rapidly with potassium amide in liquid ammonia (Note 1). The yield of aniline-1,2-$C^{14}_{1/2}$ (Note 2) is 43%. Degradation studies (Note 2) indicate that about 52% of the activity is found at C-1, as a result of rearrangement.

B. Notes

1. The reaction has been studied thoroughly by Bergstrom,[1] and a review of aromatic nucleophilic substitution reactions is given by Bunnett.[2]

2. The aniline-1,2-$C^{14}_{1/2}$ is converted to phenol-1,2-$C^{14}_{1/2}$ by diazotization. Hydrogenation over Raney nickel converts the phenol to cyclohexanol-1,2-$C^{14}_{1/2}$, which then is oxidized with dichromate to cyclohexanone-1,2-$C^{14}_{1/2}$. By means of the Schmidt reaction the ring is opened adja-

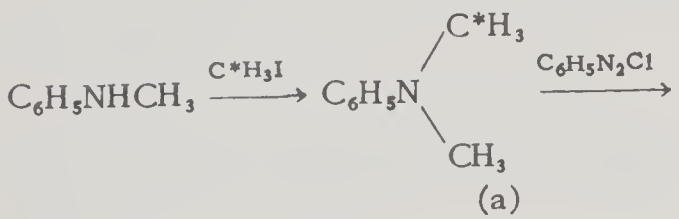
cent to the carbonyl group with formation of 6-aminohexanoic-1,2,6-$C^{14}_{1/3}$ acid. This is converted by the Schmidt reaction to carbon-C^{14} dioxide and pentamethylenediamine-1-C^{14}, which is assayed as the dibenzenesulfonamide. The molar specific activities are, respectively, 43.1% and 51.8% of that of the cyclohexanone-1,2-$C^{14}_{1/2}$ (as the semicarbazone or 2,4-dinitrophenylhydrazone). The carbon dioxide originates at C–1 of the ring (see 2-hydroxycyclohexanone-1,2-$C^{14}_{1/2}$).

In a blank degradation of aniline-1-C^{14}, from which the 1-chlorobenzene-1-C^{14} was initially made by the Sandmeyer reaction, the specific activity of the pentamethylenediamine was only 0.2 ± 1% of that of the cyclohexanone semicarbazone, thus proving the isotope location at C–1.

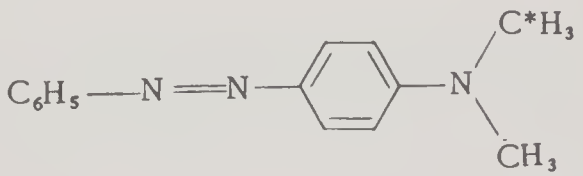
[1]F. W. Bergstrom, R. E. Wright, C. Chandler and W. A. Gilkey, J. Org. Chem., *1*, 170 (1937).

[2]J. F. Bunnett and R. E. Zahler, Chem. Revs., *49*, 273 (1951).

N,N-DIMETHYL-C^{13}_1-*p*-PHENYLAZOANILINE
(4-Dimethylamino-C^{13}_1-azobenzene)



(a)



(b)

W. S. Fones and J. White, J. Nat. Cancer Institute, *10*, 663 (1950).

A. Procedure

(a) N,N-*Dimethyl-*C^{13}_1*-aniline.* A mixture of 3.62 g. of *N*-methylaniline and 4.80 g. of methyl-C^{13} iodide is heated in a sealed tube at 100° for 17 hours. The mixture is made alkaline and steam-distilled into 20 ml. of 6 *N* hydrochloric acid (Note 1).

(b) N,N-*Dimethyl-*C^{13}_1-p-*phenylazoaniline.* The *N,N*-dimethyl-C^{13}_1-aniline is coupled with the diazonium salt prepared from 3.15 g. of aniline according to the procedure of Fones,[1] which follows. The yield, after two recrystallizations from 95% alcohol, is 4.9 g., m.p. 114–116° (cor.), 64.5% based on methyl iodide.

To a stirred mixture of 6.5 g. of aniline hydrochloride, 9 ml. of concentrated hydrochloric acid and 50 ml. of water at 0–5° is added dropwise a solution of 3.9 g. of sodium nitrite in 50 ml. of water. The solution is stirred for 30 minutes; then the excess nitrous acid is destroyed by the addition of urea (starch iodide test). To the cold, stirred solution is added 9 g. of *N,N*-dimethylaniline, followed at one-hour intervals by two portions of 7 g. of crystalline sodium acetate in 15 ml. of water. The mixture then is stirred for 4 hours at room temperature and stored overnight. The precipitated product is collected in 10.7-g. yield (95.5%), m.p. 113–116° (Note 2).

B. Notes

1. The product is not isolated, but used as an aqueous solution of the hydrochloride. In contrast to the preparation of *N,N*-dimethylaniline by methylating aniline,[1] better yields are obtained without a sodium acetate buffer to take up the hydriodic acid formed in the methylation of methylaniline. The direct methylation of *p*-phenylazoaniline leads to a mixture of compounds.[2]

2. Purified *N,N*-dimethyl-*p*-phenylazoaniline melts at 117–118°.

3. One mole of the amine is diazotized with 1 mole of sodium nitrite in concentrated aqueous solution in the presence of 2.5 moles of hydrochloric acid in 12 moles of water at 0°. The solution of diazonium salt is added to a 70% alcohol solution of the tertiary amine at 10–15° containing 2 moles of sodium acetate. The crude product is recrystallized from benzene-petroleum ether.

C. Other Preparations

N,N-Dimethyl-C_1^{14}-*p*-(*m*-tolylazo)aniline, (3′-methyl-4-dimethylamino-C_1^{14}-azobenzene), is prepared[3] in a similar manner,[4] by coupling *N,N*-dimethyl-C_1^{14}-aniline with a large excess of *m*-toluenediazonium chloride (Note 3). Purification is accomplished by chromatography (twice in petroleum ether) on alumina[5] and elution with benzene; yield 38% based on methyl iodide.

N,N-Dimethyl-C_1^{14}-aniline has been prepared in a similar manner by Miller.[3] A mixture of 1 mmole of methyl-C^{14} iodide and 3 mmoles of *N*-methylaniline in ether was stored at room temperature for 17 hours, and then heated to 60° for 10 minutes. The product was separated by the Hinsberg reaction.

N,N-Dimethyl-C_1^{14}-*p*-phenylazoaniline has been prepared by Boissonnas,[2] in 43.5% yield based on 1.9 mmoles of methanol. *N*-Methyl-*p*-phenylazoaniline was alkylated with methyl-C^{14} iodide in the presence of sodium in liquid ammonia.

N,N-Dimethyl-C_2^{14}-*p*-phenylazoaniline and *N*-methyl-C^{14}-*p*-phenylazoaniline have been prepared by MacDonald,[6] by heating 0.33 mmole of methyl-C^{14} iodide with 1.3 mmoles of *p*-phenylazoaniline in a sealed, 1-ml. Pryex tube at 50° for 7.5 hours. The contents were dissolved in alcohol containing a slight excess of ammonia, and the solution was evaporated to dryness. The mixture of primary, secondary and tertiary amines was resolved by chromatography on a 1 × 24-inch column of alumina.[7] *N*-Methyl-C^{14}-4-phenylazo-*o*-toluidine, *N,N*-dimethyl-C_2^{14}-4-phenylazo-*o*-toluidine, *N*-methyl-C^{14}-*p*-(*p*-tolylazo)aniline and *N,N*-dimethyl-C_2^{14}-*p*-(*p*-tolylazo)aniline have been prepared in a similar manner (heating 27 hours), starting with 4-phenylazo-*o*-toluidine or *p*-(*p*-tolylazo)aniline, respectively. The combined radiochemical yield of the six methylated dyes was 78%. Directions are given by MacDonald[6] for preparing the starting materials. The products were identified by chromatography on alumina (alone and in mixture with authentic known samples), by their characteristic colors in acid solution,[5,8] and by the melting points.[4]

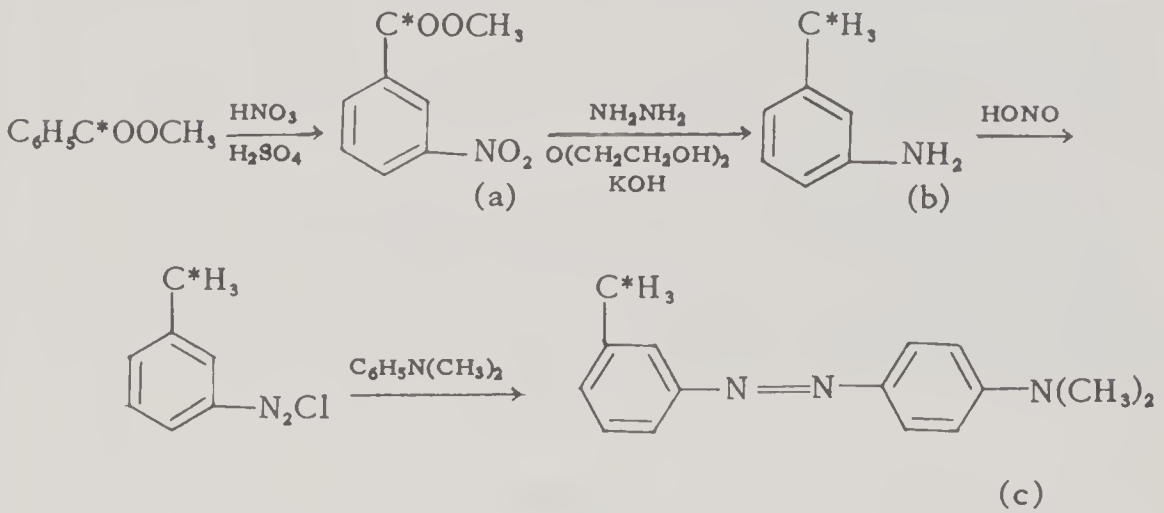
The preparation of *N,N*-dimethyl-*p*-phenyl-1-C^{14}-azoaniline has been described by Hromatka.[9] To a stirred, ice-cooled solution of 8.073 g. of aniline-1-C^{14} hydrochloride and 1 ml. of 2 *N* hydrochloric acid is added dropwise 0.124 ml. of 0.5 *N* sodium nitrite, followed 5 minutes later by 0.094 ml. (80.13 mg./ml.) of *N,N*-dimethylaniline in 2 *N* hydrochloric acid. After 1 hour of stirring, the mixture is treated with 1.0 g. of sodium acetate, stirred for 30 minutes and filtered. The product is washed with 3 ml. of water, dissolved in 6 ml. of carbon tetrachloride and chromatographed on 8.3 g. of alumina (8.5 cm.). The upper band is discarded mechanically, and the colored product band is eluted with methanol-carbon tetrachloride (1:9); yield (determined colorimetrically) 0.0133 g. (94.8%).

The preparation of dodecyl-1-C^{14}-trimethylammonium bromide has been reported by Jerchel.[10] A mixture of 2.15 g. of 1-bromododecane-1-C^{14} and an excess of 25% alcoholic trimethylamine was heated at 120° for 12 hours, and the product was precipitated with ether and recrystallized from absolute alcohol-ether; yield 54.2%, m.p. 217–219°.

[1]W. S. Fones and J. White, Arch. Biochem., *20*, 118 (1949).
[2]R. A. Boissonnas, R. A. Turner and V. du Vigneaud, J. Biol. Chem., *180*, 1053 (1949).
[3]E. C. Miller, A. M. Plescia, J. A. Miller and C. Heidelberger, J. Biol. Chem., *196*, 863 (1952).
[4]J. A. Miller and E. C. Miller, J. Exp. Med., *87*, 139 (1948).
[5]J. A. Miller and C. A. Baumann, Cancer Research, *5*, 157 (1945).
[6]J. C. MacDonald, A. M. Plescia, E. C. Miller and J. A. Miller, Cancer Research, *13*, 292 (1953).
[7]J. A. Miller and C. A. Baumann, Cancer Research, *5*, 157 (1945).
[8]J. A. Miller, R. W. Sapp and E. C. Miller, J. Am. Chem. Soc., *70*, 3458 (1948).

[9]O. Hromatka and L. H. Schlager, Monatsh., *85*, 29 (1954).
[10]D. Jerchel, H. Becker and K. Schmeiser, Z. Naturforsch., *9b*, 169 (1954).

N,N-DIMETHYL-*p*-(*m*-TOLYLAZO-α-C^{14})ANILINE
(3-Methyl-C^{14}-4-dimethylaminoazobenzene)



D. A. Salzberg, W. Nye, A. C. Griffin, Arch. Biochem. Biophys., *27*, 243 (1950).

A. Procedure

(a) *Methyl* m-*Nitrobenzoate-C^{14}*. Methyl benzoate-C^{14} is nitrated in 50% yield (5.4 g.), by the procedure of Kamm and Segur.[1]

(b) m-*Toluidine-α-C^{14}* (Note 1). To a solution of 2.0 g. of methyl *m*-nitrobenzoate-C^{14} in 12 ml. of diethylene glycol, in a 200-ml. flask, is added a concentrated aqueous solution containing 3 ml. of 85% hydrazine hydrate and 2 g. of potassium hydroxide. The flask is attached to a 20-cm. Vigreux column fitted with a side arm extending to the bottom of an ice-cooled receiver, and the mixture is refluxed for 10 minutes, then distilled until there is a sudden rise in distillation temperature above the boiling point of water. The product is extracted from the distillate with ether. Evaporation of the solvent leaves the product in 30% yield (Note 2); the molar specific activity is the same as that of the starting material.

(c) N,N-*Dimethyl*-p-(m-*tolylazo-α-C^{14})aniline* (Note 3). The *m*-toluidine-α-C^{14} is diazotized[2] with 1 mole of sodium nitrite in the presence of 2.5 moles of hydrochloric acid at 0°; then the solution of *m*-toluenediazonium chloride is added to a 70% ethanol solution containing 1 mole of *N,N*-dimethylaniline and 2 moles of sodium acetate. The insoluble product is collected and recrystallized from ethanol-water.

B. Notes

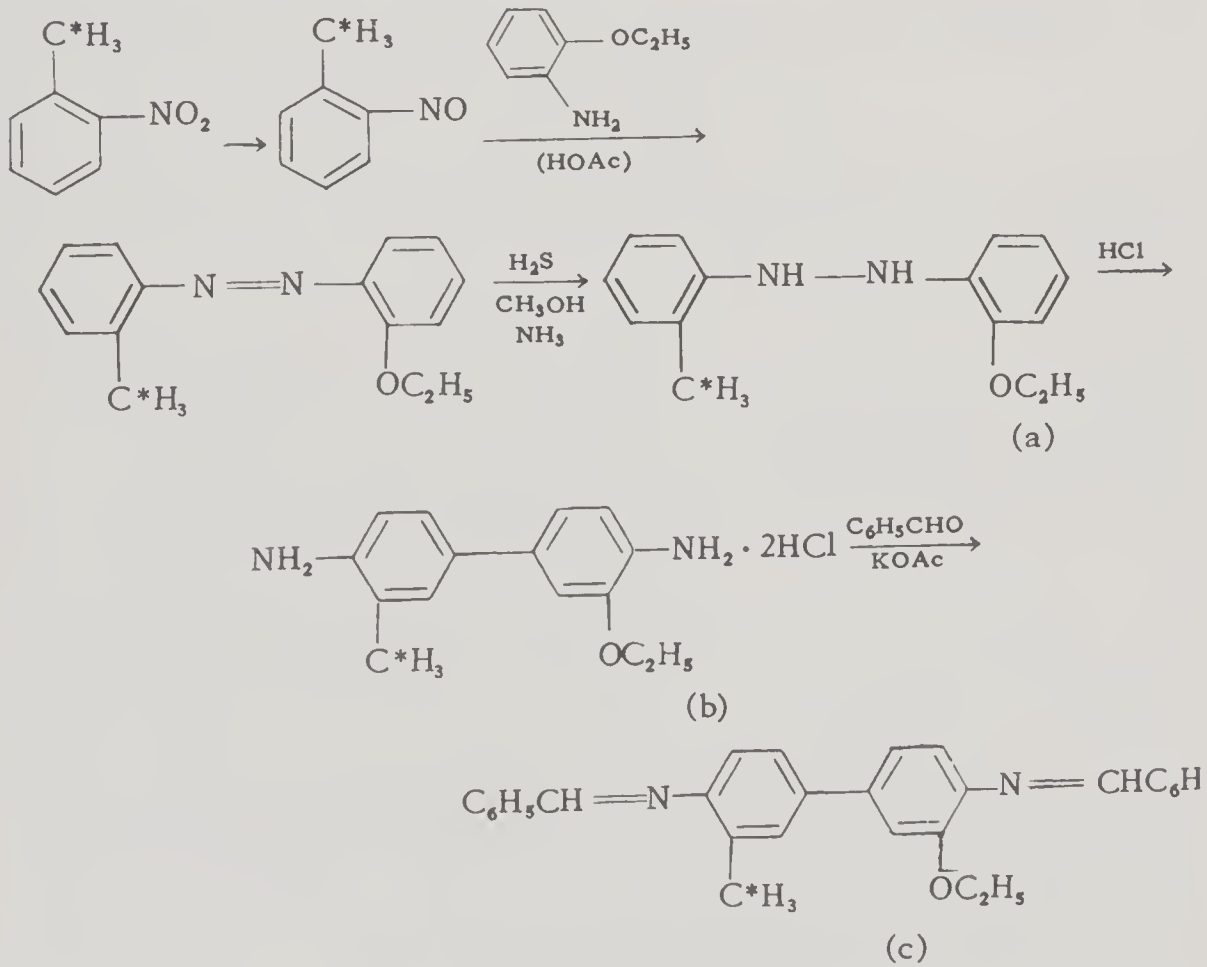
1. The procedure is that described by Salzberg,[3] combining the McFadyen and Stevens[4] reduction of esters to aldehydes with the Huang-Minlon[4] reduction of nitrobenzaldehydes to toluidines. The method seems to be successful only with *m*-nitrobenzoic esters. High-pressure hydrogenation fails to give the desired product.

2. The compounds boils at 201° (756 mm.) and the *m*-acetotoluidide, formed by reaction with acetic anhydride, melts at 65°.

3. The procedure is according to the method of Miller;[5] also see *N*,*N*-dimethyl-C_1^{13}-*p*-phenylazoaniline.

[1]*Organic Snytheses*, Coll. Vol. I, Wiley, New York, 1941, p. 372.
[2]*Organic Syntheses*, Vol. 27, Wiley, New York, 1947, p. 81.
[3]D. A. Salzberg, J. Am. Chem. Soc., *72*, 4307 (1950).
[4]Huang-Minlon, J. Am. Chem. Soc., *70*, 2802 (1948); J. S. McFadyen and T. S. Stevens, J. Chem. Soc., *1936*, 584.
[5]J. A. Miller and C. A. Baumann, Cancer Research *5*, 227 (1945).

N,*N*′-DIBENZYLIDENE-3-ETHOXY-3′-METHYL-C^{14}-BENZIDINE



D. H. Smith, J. R. Schwartz and G. W. Wheland, J. Am. Chem. Soc., *74*, 22[illegible] (1952); G. W. Wheland and J. R. Schwartz, J. Chem. Phys., *17*, 425 (1949).

A. Procedure (Note 1)

(a) *2-Ethoxy-2′-methyl-C^{14}-hydrazobenzene.* o-Nitrotoluene-α-C^{14} is converted to o-nitrosotoluene-α-C^{14}, which is condensed with o-phenetidine in ligroin (or ethanol) containing about 2% acetic acid. The 2-ethoxy-2′-methyl-C^{14}-azobenzene, m.p. 70–71°, is reduced to the product with hydrogen sulfide in alcoholic ammonia, m.p. 95–97°.

(b) *3-Ethoxy-3′-methyl-C^{14}-benzidine Dihydrochloride.* To a stirred solution of 5.6 mmoles of the hydrazo compound in 50 ml. of absolute ether is added the calculated amount of 1.7 *N* hydrogen chloride in absolute ethanol, followed by 50 ml. of ether. The product is collected, washed with ether and dried in a desiccator for 24 hours (Note 2).

(c) N,N′-*Dibenzylidene-3-ethoxy-3′-methyl-C^{14}-benzidine.* A mixture of the dihydrochloride, 45 ml. of absolute alcohol, 1.0 ml. of benzaldehyde and 1.4 g. of potassium acetate is refluxed for 20 hours. The crude product is collected and washed with two 3-ml. portions of cold ethanol, two 5-ml. portions of cold water and alcohol (Note 3).

B. Notes

1. The preparations were incidental to a study establishing the intramolecular character of the rearrangement of hydrazobenzenes to 4,4′-diaminobiphenyls in the presence of strong acids (benzidine rearrangement).[1] No radioactivity could be found by isotopic dilution of the product with carrier cross-products of the reaction.

2. Benzidine free bases may be isolated by treating an aqueous solution of the hydrochloride with sodium acetate and extracting continuously with ether. Purification may be accomplished by recrystallization from alcohol containing a little ammonium sulfide.[1]

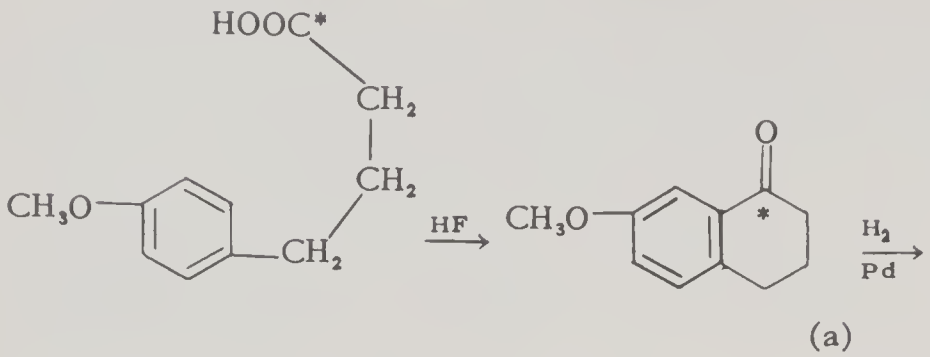
3. Purification of the Schiff base may be accomplished by recrystallization, or chromatography in benzene solution on alumina and elution with benzene-ether.

C. Other Preparations

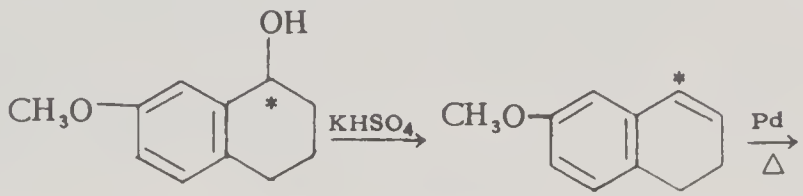
By similar procedures from o-nitrosotoluene-α-C^{14} and aniline are prepared: 2-methyl-C^{14}-hydrazobenzene, 3-methyl-C^{14}-benzidine dihydrochloride, and *N,N′*-dibenzylidene-3-methyl-C^{14}-benzidine (67% yield based on the hydrazobenzene).

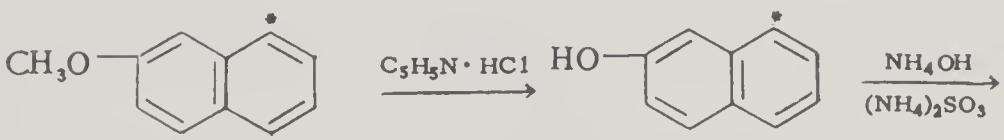
[1]C. K. Ingold and H. V. Kidd, J. Chem. Soc., *1933*, 984; P. Jacobson, Ann., *428*, 76 (1922).

2-NAPHTHYLAMINE-8-C^{14}

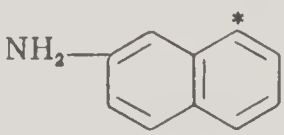


(a)





(b) (c)



(d)

J. R. Catch, H. P. W. Huggill and A. R. Somerville, J. Chem. Soc., *1953*, 3028.

A. Procedure

(a) *3,4-Dihydro-7-methoxy-1*(2H)*-naphthalenone-1-*C^{14}, (*7-Methoxy-1-tetralone-1-*C^{14}) (Note 1). A mixture of 0.493 g. of 4-(*p*-methoxyphenyl)butyric-1-C^{14} acid and 5 ml. of anhydrous hydrogen fluoride is kept stoppered overnight in a cooled "polythene" tube. The solvent is evaporated, and residue is neutralized with sodium carbonate solution and extracted with ether. The extract is washed with sodium carbonate solution and evaporated to dryness. The residual yellow crystals (0.403 g., 90%) are dissolved in ethereal diazomethane, to methylate any free phenol; then the solution is evaporated to dryness.

(b) *2-Methoxynaphthalene-8-C^{14}* (Note 2). The 7-methoxy-1-tetralone-1-C^{14} is hydrogenated in methanol over 0.050 g. of palladium-black until one molecular amount of hydrogen has been absorbed (Note 3). The filtered solution is evaporated, and the residue is heated for 2 hours at 200° with 0.3 g. of freshly fused potassium bisulfate. The melt is extracted with ether, and the extract is washed with water, dried and evaporated. The residue is mixed with 0.05 g. of palladium-black, and heated for 2.5 hours at 200–230°. The mixture of product and naphthalene-8-C^{14} is distilled at about 60° (10^{-2}mm.); yield (crude) 0.288 g.

(c) *2-Naphthol-8-C^{14}*. The crude 2-methoxynaphthalene-8-C^{14} is mixed with ten times its weight of pyridine hydrochloride and heated for 6 hours at 200° (Note 4). The crude product is extracted into ether, which is then washed with dilute hydrochloric acid and extracted with sodium hydroxide solution (Note 5). The alkaline solution is acidified and extracted with ether; evaporation gives 0.135 g. of crude product (Note 6).

(d) *2-Naphthylamine-8-C^{14}* (Note 7). The crude 2-naphthol-8-C^{14} (0.135 g.) is heated for 17 hours at 150° in a sealed Pyrex tube (20 × 200-mm.), which is half-filled with equal parts of concentrated ammonium hydroxide and freshly prepared ammonium sulfite solution. The mixture is extracted with ether, and the extract is washed with water and extracted with dilute hydrochloric acid. The acid solution is made basic and extracted with ether. Evaporation gives a crude yield of 0.101 g. (Note 6). Purification is effected by six sublimations at 80–95° at 10^{-6} mm. from powdered silica (10% by weight, 100–200 mesh) in a long tube with constrictions, which are successively sealed off. The colorless product melts at 108.4–109.9° (Note 8).

B. Notes

1. The procedure is adapted from the method of Campbell and Todd;[1] also see 2-hydroxy-9-anthrone-9-C^{14}. By the same procedure, from 4-phenylbutyric-1-C^{14} acid is prepared[2] 3,4-dihydro-1(2*H*)-naphthalenone-1-C^{14}, (1-tetralone-1-C^{14}); yield 90%.

2. Heating 1-tetralone-1-C^{14} with palladium-charcoal for 5 hours in a sealed tube at 270° gives a 72% yield of naphthalene-1-C^{14} and a 10% yield of 1-naphthol-1-C^{14}. A Clemmensen reduction of 3,4-dihydro-1(2*H*)-naphthalenone-1-C^{14}, followed by heating the 1,2,3,4-tetrahydronaphthalene-1-C^{14} for 19 hours at 215° with Raney nickel gives an 85% yield of naphthalene-1-C^{14}.

3. Hydrogenation is slow and the addition of fresh catalyst may be advisable. The direct reduction to the tetralin is uncertain and dehydrogenation then produces up to 40% naphthalene itself. The method indicated is most satisfactory.

4. The ether hydrolysis is adapted from the method of Prey[3] (see *N*-methyl-C^{14}-normorphine). The ordinary commercial salt contains a little pyridine which is desirable.

5. Evaporation of the ether solution yields 0.137 g. of naphthalene-1-C^{14}, m.p. 76–79°; picrate, m.p. 149–150°. The melting point is raised to 79.5° by chromatography on alumina and crystallization from alcohol.

6. The product is also assayed as the acetyl derivative.

7. The preparation is by the Bucherer reaction,[4] which gives a yield of 94–96%, m.p. 111–112°, from 1 mole of pure 2-naphthol.

8. 2-Naphthylamine is stable in the absence of light and oxygen, dibenzo[b,i]phenazine-4,11-C_2^{14} probably being formed by air oxidation.[5] Bis(2-naphthyl-8-C^{14})amine is probably produced during the Bucherer reaction as a by-product.

[1]W. P. Campbell and D. Todd, J. Am. Chem. Soc., 64, 928 (1942).

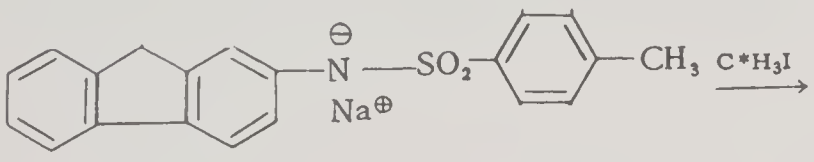
[2]R. J. Speer, D. R. Carmody and M. L. Humphries, Nucleonics, 7(4), 48, 47 (1950).

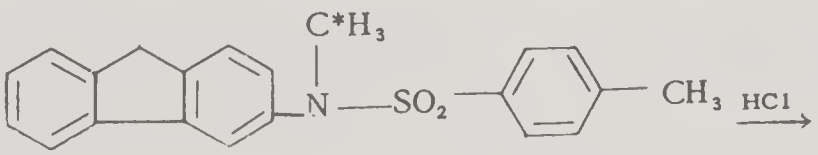
[3]V. Prey, Ber., 74, 1219 (1941).

[4]*Organic Reactions*, Vol. I, Wiley, New York, 1942, p. 105.

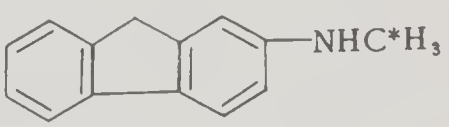
[5]A. F. Henson, A. R. Somerville, M. E. Farquharson and M. W. Goldblatt, Biochem. J., 58, 383 (1954).

N-METHYL-C^{14}-2-FLUORENAMINE





(a)



(b)

J. N. Little and F. E. Ray, J. Am. Chem. Soc., 74, 4955 (1952).

A. Procedure (Note 1)

(a) N-*2-Fluorenyl*-N-*Methyl*-C^{14}-p-*toluenesulfonamide*. To a mixture of 0.715 g. (2 mmoles) of freshly prepared sodium *N*-2-fluorenyl-*p*-toluenesulfonamide (Note 2) and acetone is added 0.1419 g. (1 mmole) of methyl-C^{14} iodide, by vacuum technique, and the mixture is stirred for 4 hours. Non-isotopic methyl iodide (0.25 ml., 4 mmoles) is added, and the mixture is stirred for 4 hours, then diluted with water, and the product is collected and washed by centrifuging (Note 3).

(b) N-*Methyl*-C^{14}-*2-fluorenamine*, (*2-Methyl*-C^{14}-*aminofluorene*). The N-2-fluorenyl-*N*-methyl-C^{14}-*p*-toluenesulfonamide is hydrolyzed by heating for 1 hour at 165° with 50% hydrochloric acid in a sealed tube (Note 4). The mixture is removed with warm water and evaporated to dryness. The *N*-methyl-C^{14}-2-fluorenamine hydrochloride is decolorized in water-alcohol solution with charcoal. The filtrate is cooled and neutralized with ammonium hydroxide, then the product is collected (without delay), washed several times with water and dried in a desiccator in the dark (Note 5). The yield is 0.35 g. (90%), m.p. 68–70° (Note 6); the radiochemical yield is 89.5%.

B. Notes

1. This is the first synthesis of *N*-methyl-2-fluorenamine to be found in the literature.

2. Directions are given by Little for preparing the starting material in excellent yield from *N*-2-fluorenyl-*p*-toluenesulfonamide[1] and sodium hydride.

3. The yield of a trial run in 50% alcohol solution was 99.6%, m.p. 134–136°.

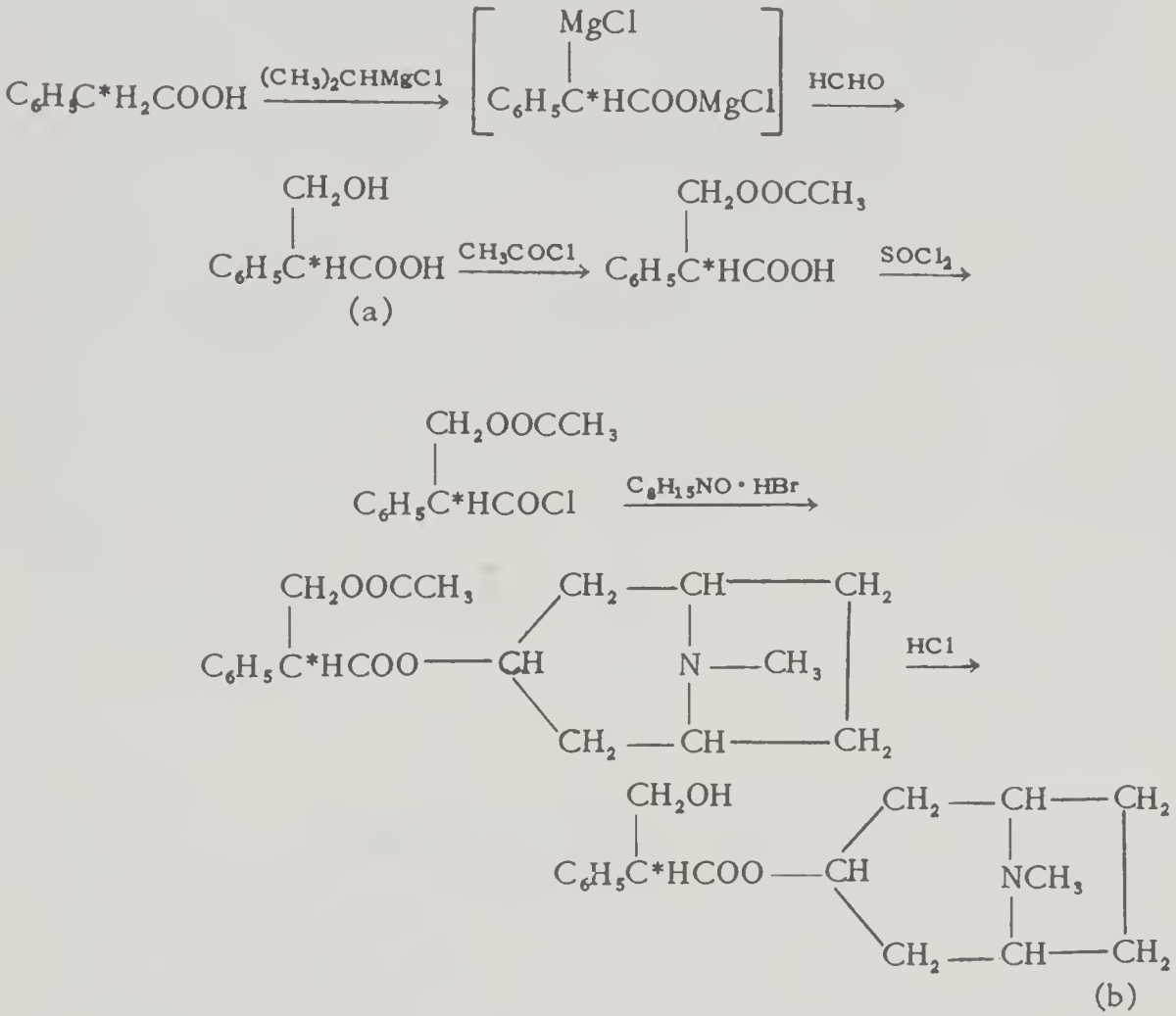
4. An 85-ml. Carius tube with 0.7 g. of compound and 20 ml. of acid was used in a trial run. The best yield is obtained by heating the tube in a horizontal position with occasional shaking. Whether the acid is 6 *N* or fuming hydrochloric acid is not clear.

5. The colorless flocculent precipitate darkens upon standing.

6. One recrystallization from ethanol-water raises the melting point to 73–75°. The pure compound melts at 76–77°.

[1]N. Campbell, W. Anderson and J. Gilmore, J. Chem. Soc., *1940*, 446.

3-(8-METHYL-8-AZABICYCLO[3.2.1]OCTYL) TROPATE-α-C^{14} (C_1^{14}-Atropine)



J. A. Montgomery and R. E. Burks, Jr., Southern Research Institute, Birmingham, Alabama, private communication.

A. Procedure

(a) *Tropic-α-C^{14} Acid, (2-Phenylhydracrylic-2-C^{14} Acid)* (Note 1). To a stirred Grignard solution, prepared from 2.92 g. (37.2 mmoles) of isopropyl chloride, is added slowly 1.65 g. of phenylacetic-2-C^{14} acid in benzene. When the spontaneous reaction subsides, the mixture is refluxed for 2 hours. The gaseous formaldehyde generated from 1 g. of paraformaldehyde (Note 2) is passed into the stirred mixture with ice-bath cooling. The mixture is hydrolyzed by pouring onto ice and sulfuric acid; then the mass is stirred and filtered, and the ether layer is separated. The aqueous layer and solid material are digested, and thoroughly extracted with ether. Evaporation leaves 0.863 g. of crude product, which is recrystallized from ben-

zene; yield 0.632 g., m.p. 109–110° (Note 3). The molar specific activity agrees with that of the starting material.

(b) *3-(8-Methyl-8-azabicyclo[3.2.1]octyl) 2-Phenylhydracrylate-2-C^{14}* (Note 4). To 0.319 g. of tropic-α-C^{14} acid is added 0.5 ml. of acetyl chloride. When the evolution of gas ceases, the mixture is warmed to 70–80° for 15 minutes in such a manner that the excess acetyl chloride distills off. The excess thionyl chloride is vacuum-distilled (at the pressure of a water aspirator) into a trap cooled with Dry Ice, followed by distillation of a portion of added dry benzene. To the residual 3-acetoxy-2-phenylpropionyl-2-C^{14} chloride is added 0.500 g. of tropine hydrobromide, and the stirred mixture is heated from 70° to 100° over the course of 1 hour. The mixture is heated for 1 hour at 100° with 2 ml. of water containing 1 drop of concentrated hydrochloric acid, then cooled, and neutralized with dilute sodium hydroxide. The oily precipitate slowly crystallizes, and the product, after two precipitations from chloroform solution with petroleum ether, melts at 105–108° (Note 5); yield 0.1258 g. The molar specific activity is the same as that of the tropic-α-C^{14} acid.

B. Notes

1. The procedure is adapted from the method of Blicke, Raffelson and Barna.[1]

2. Paraformaldehyde, dried for 2 days in a desiccator over phosphorus pentoxide, is heated in a distilling flask at 180–200°. The formaldehyde is carried into the vigorously stirred mixture by a slow stream of dry nitrogen.

3. Recrystallization of one-half of this material gives 0.2357 g. of pure tropic-α-C^{14} acid, m.p. 115–116°. The compound is chromatographically homogeneous.[2]

4. The procedure is an adaptation of the method of Wolffenstein and Mamlock.[3]

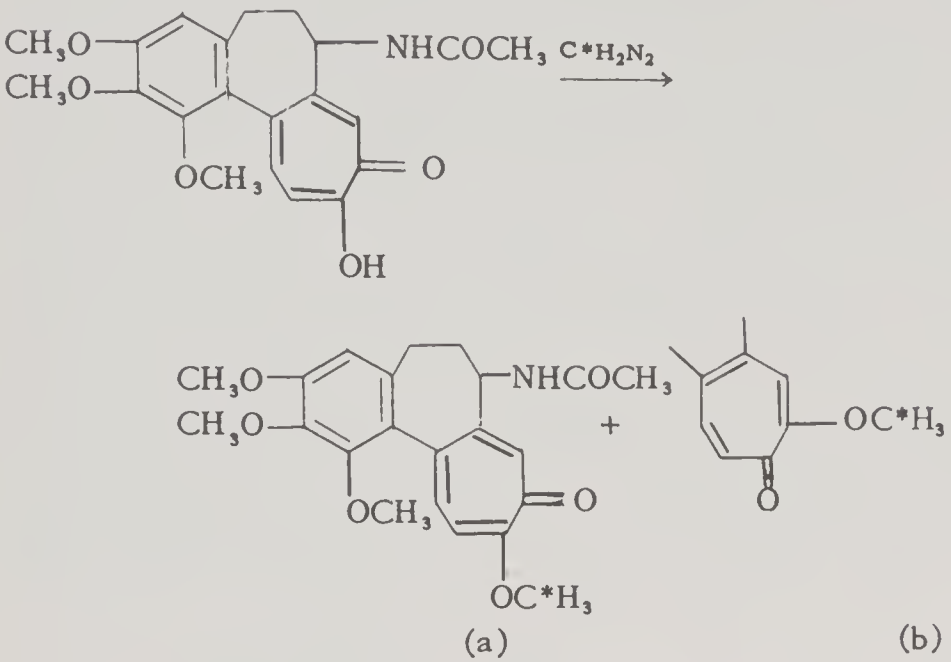
5. The melting point is unchanged by sublimation and an additional precipitation from chloroform solution. Assay by a modification of the colorimetric method of Vitali-Moran indicates[2] that the chemical purity is 85%. The compound contains 7% of a radio-impurity indistinguishable chromatographically from the product, and which is removed by a series of chloroform extractions.[2]

[1]F. F. Blicke, H. Raffelson and B. Barna, J. Am. Chem. Soc., *74*, 253 (1952).

[2]R. E. Gosselin, J. D. Gabourel, S. C. Kalser and J. H. Wills, J. Pharmacol. Exptl. Therap., *115*, 217 (1955).

[3]R. Wolffenstein and L. Mamlock, Ber., *41*, 723 (1908).

7-ACETAMIDO-6,7-DIHYDRO-1,2,3-TRIMETHOXY-10-METHOXY-C^{14}-BENZO[*a*]HEPTALEN-9(5*H*)-ONE (C_1^{14}-Colchicine)



R. F. Raffauf, A. L. Farren and G. E. Ullyot, J. Am. Chem. Soc., 75, 2576 (1953).

A. Procedure (Note 1)

To a solution of 0.550 g. (1.43 mmoles) of colchiceine in about 20 ml. of methylene chloride, cooled with a Dry Ice-bath, is added a mixture of ether and 2.00 mmoles of diazomethane-C^{14} (Note 2) by distillation. The mixture is stored overnight at 0–5°; then the excess of diazomethane-C^{14} is distilled into a receiver cooled by Dry Ice (Note 3). The solvents are distilled under vacuum, and a chloroform solution of the residue is transferred to a column of 24 g. of neutral alumina (Note 4), the chromatogram being developed with 80-ml. portions of solvent. Elution by chloroform-0.5% methanol gives two fractions which, after evaporation and recrystallization from ethyl acetate-ether, yield 0.310 g. of C_1^{14}-colchicine (a), m.p. 155–160°, and 0.250 g. of a mixture of colchicine and C_1^{14}-isocolchicine (b), m.p. 155–170° (Note 5). Elution with chloroform-1% methanol gives 0.030 g. of C_1^{14}-isocolchicine, (1,2,3-trimethoxy-9-methoxy-C^{14}-7-acetamido-5,6,7,7a,12a-pentahydrobenzo[*a*]heptalen-10-one), m.p. 216–218°. The combined residue from the evaporated mother liquors is 0.130 g.

B. Notes

1. Some reactions of ring C are presented by Horwitz and Ullyot.[1]

2. As the usual methods[2] for assay of diazomethane consume the reagent, giving unwanted labeled compounds, a sensitive micro-colorimetric method was developed.[3] A solution of diazomethane is allowed to react

with an excess of colchiceine, and the excess, as the ferric chloride complex, is measured colorimetrically by comparison with standard colchiceine solutions (1-0.1 micromole/10 ml.).

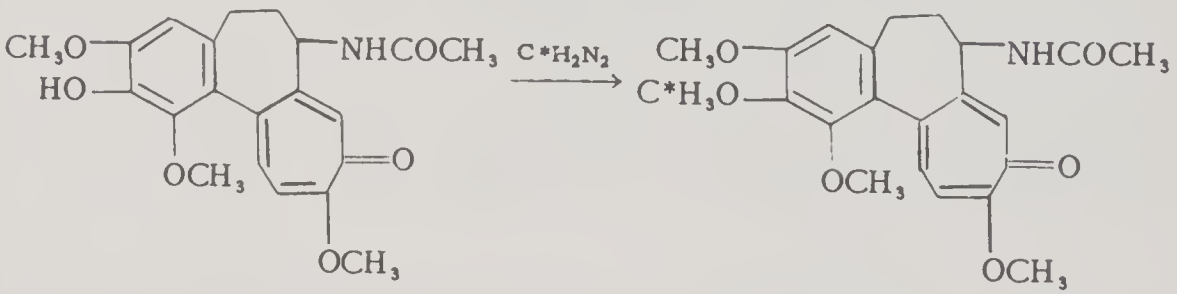
3. The methylated mixture gives a negative color test with ferric ion.

4. The alumina is washed free of alkali with hot water, then alcohol, and dried under vacuum at 185° for 30 minutes. The chloroform is freed from preservative ethanol.

5. The isomers resulting from this reaction were first separated by Sorkin.[4]

[1]R. M. Horowitz and G. E. Ullyot, J. Am. Chem. Soc., *74*, 587 (1952).
[2]E. K. Marshall, Jr. and S. F. Acree, Ber., *43*, 2323 (1910).
[3]E. Boyland and E. H. Mawson, Biochem. J., *32*, 1204 (1938); R. F. Raffauf, A. L. Farren and G. E. Ullyot, J. Am. Chem. Soc., *75*, 2576 (1953).
[4]M. Sorkin, Helv. Chim. Acta, *29*, 246 (1946).

7-ACETAMIDO-6,7-DIHYDRO-1,3,10-TRIMETHOXY-2-METHOXY-C^{14}-BENZO[*a*]HEPTALEN-9(5*H*)-ONE (C_1^{14}-Colchicine)

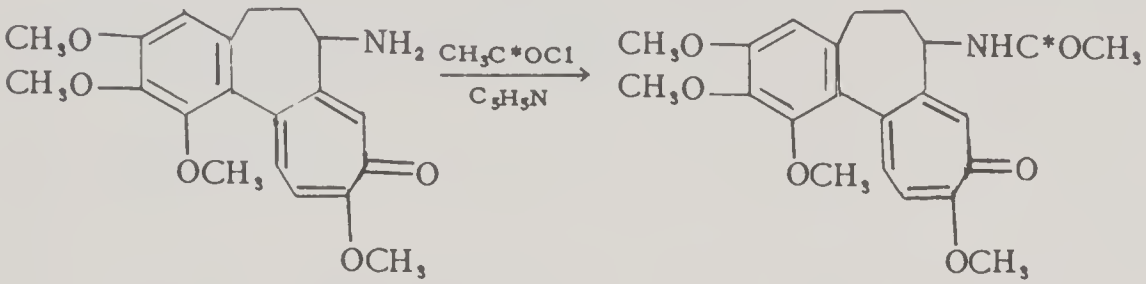


R. F. Raffauf, A. L. Farren and G. E. Ullyot, J. Am. Chem. Soc., 75, 2576 (1953).

Procedure

To a solution of 7.75 mmoles of diazomethane-C^{14} in ether is added a solution of 0.770 g. of demethylcolchicine in a little methylene chloride containing 10% methanol. The flask is fitted with a cold-finger reflux condenser, cooled with Dry Ice-acetone, while a trap containing ether at −78° guards the outlet of the condenser. The mixture is heated under gentle reflux for 1 hour, then the excess of diazomethane-C^{14} is recovered by distillation. The reaction mixture is evaporated to dryness under reduced pressure. A solution of the residue in chloroform-0.5% methanol is purified by passage through a small column (1 × 10 cm.) of slightly alkaline alumina. Evaporation of solvent and recrystallization of the residue from ethyl acetate-ether yield 0.510 g. of product, m.p. 154–158°. The mother liquors contain 0.180 g. of solids, and the column contains a small amount of colored by-product.

7-ACETAMIDO-1-C^{14}-6,7-DIHYDRO-1,2,3,10-TETRAMETHOXY-BENZO[*a*]HEPTALEN-9(5*H*)-ONE
(C_1^{14}-Colchicine)

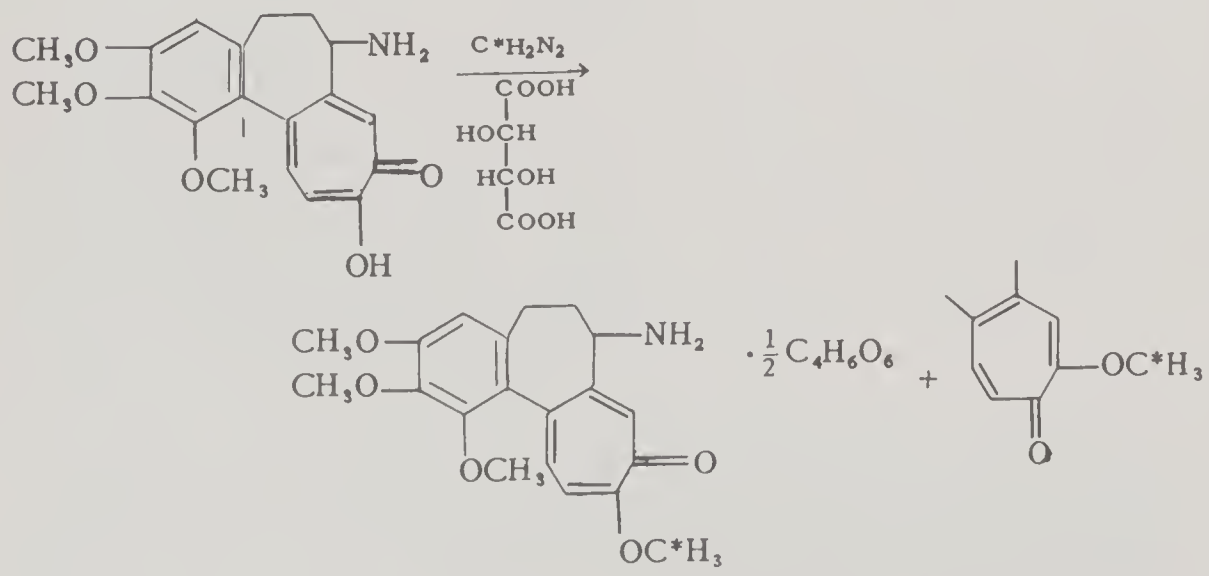


R. F. Raffauf, A. L. Farren and G. E. Ullyot, J. Am. Chem. Soc., 75, 2576 (1953).

Procedure

To a mixture of 1.0 g. (2.8 mmoles) of *n*-trimethylcolchicinic acid methyl ether and pyridine is added, by vacuum technique, 3.5 mmoles of acetyl-1-C^{14} chloride. The flask is closed off, and the mixture is warmed to 55–60° for 15 minutes; then the solvents are removed by distillation. The residue is dissolved in chloroform and washed with water, and the solvent is again removed under vacuum. The crude product (1.060 g.) is dissolved in chloroform-0.5% methanol and passed through a column of neutral alumina (1 × 10 cm.). Evaporation of solvent and recrystallization of the residue from ethyl acetate-ether yields 0.780 g. of product, m.p. 153–155°. The mother liquors contain 0.130 g. of solids.

7-AMINO-6,7-DIHYDRO-1,2,3-TRIMETHOXY-10-METHOXY-C^{14}-BENZO[*a*]HEPTALEN-9(5*H*)-ONE D-TARTRATE
(*n*-Trimethylcolchicinic Acid Methyl-C^{14} Ether D-Tartrate)



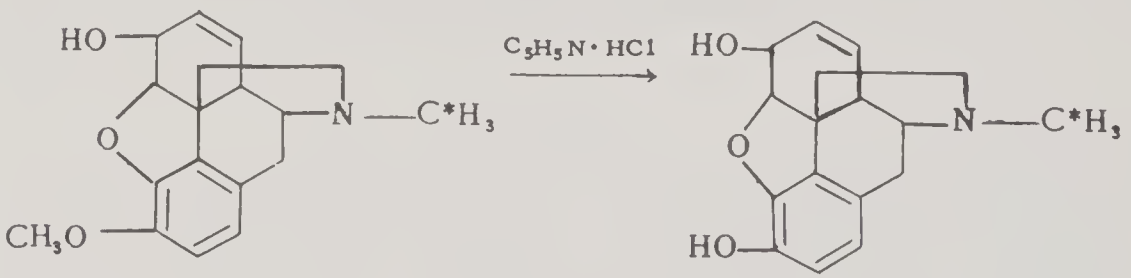
R. F. Raffauf, A. L. Farren, and G. E. Ullyot, J. Am. Chem. Soc., 75, 2576 (1953).

Procedure

A mixture of diazomethane-C^{14} in ether, and excess trimethylcolchicinic acid in methylene chloride is set aside for 4 hours at 0–5°. The mixture is evaporated under vacuum, and the residue is taken up in 0.1 *N* sodium hydroxide and twice extracted with chloroform. The chloroform extract is washed with water and evaporated to dryness under vacuum. In an ethanol solution of the residue is dissolved a slight excess of D-tartaric acid, and the solution is stored overnight at room temperature. The product is collected, washed with alcohol, and dried; yield 0.630 g., m.p. 206–208° (dec.). A sample recrystallized from dilute alcohol melts at 213–216° (dec.).

Concentration of the mother liquors and crystallization from methanol-acetone yield 0.250 g. of crude trimethylisocolchicinic acid methyl-C^{14} ether D-tartrate, 7-amino-6,7-dihydro-1,2,3-trimethoxy-9-methoxy-C^{14}-benzo[*a*]heptalen-10(5*H*)-one D-tartrate, m.p. 160–170° (dec.).

N-METHYL-C^{14}-NORMORPHINE
(C_1^{14}-Morphine)



H. Rapoport, C. H. Lovell and B. M. Tolbert, J. Am. Chem. Soc., 73, 5900 (1951).

A. Procedure

A mixture of 1.00 g. of *N*-methyl-C^{14}-norcodeine and about 3 g. of pyridine hydrochloride is heated for 6 minutes in a nitrogen atmosphere with a bath at 220° (Note 1). The mixture is immediately cooled, dissolved in 20 ml. of water, and basified with 10 ml. of 4 *N* sodium hydroxide. The non-phenolic material is removed by extraction with four 15-ml. portions of chloroform, which are then washed with 10 ml. of 0.5 *N* sodium hydroxide and 10 ml. of water. The combined aqueous solution is adjusted to pH 9 with hydrochloric acid and chilled to precipitate the product, which is collected and dried. The crude product is digested with 75 ml. of hot methanol, and the mixture is filtered. The filtrate is chromatographed on an alumina column (Note 2), and eluted with 700 ml. of methanol. After evaporation of solvent, the residue is dissolved in 10 ml. of 0.2 *N* sodium hydroxide and reprecipitated at pH 9. The dried product is sublimed at 180–190° (0.1 mm.), and the sublimate is recrys-

tallized from absolute alcohol. The yield is 0.210 g. (22%), m.p. 254–255°, and the molar specific activity is the same as that of the starting material.

B. Notes

1. This procedure is based upon a mild ether cleavage reaction that has been successfully applied to a number of codeine derivatives by Rapoport and Bonner.[1] The *N*-methyl-C^{14}-norcodeine is readily prepared in the manner described by von Braun,[2] employing methyl-C^{14} iodide (see C_1^{14}-codeine hydrochloride, Note 1). Speyer and Walcher[3] have also described a method for preparing similar *N*-demethyl starting materials.

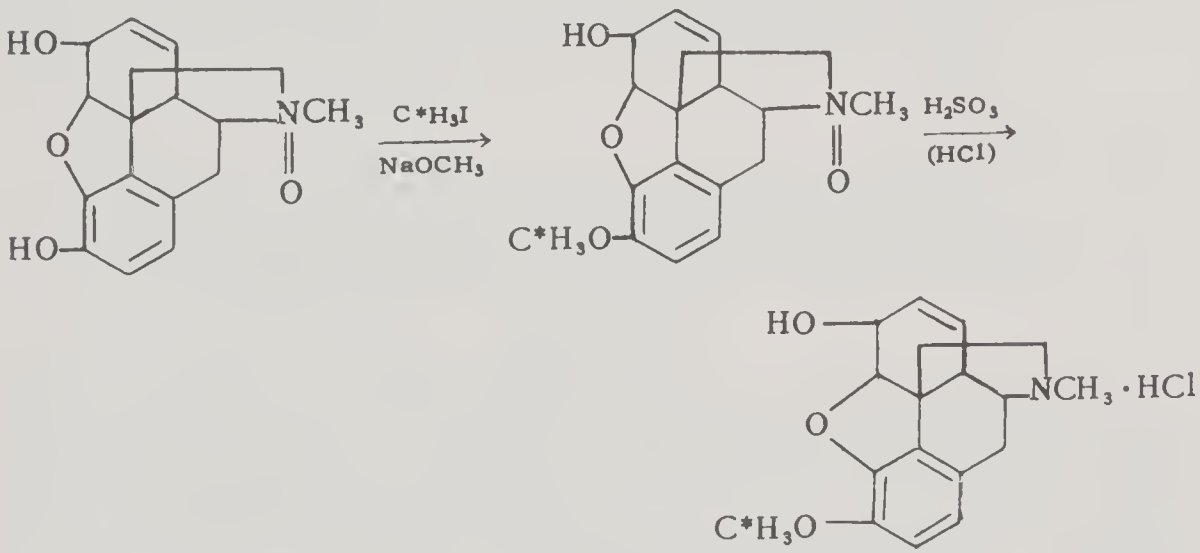
2. The column is 120 × 11 mm.

[1]H. Rapoport and R. M. Bonner, J. Am. Chem. Soc., 73, 5485 (1951); 73, 2872 (1952).

[2]J. von Braun, Ber., 47, 2312 (1914); J. Chem. Soc., 106 (1), 1138 (1914).

[3]E. Speyer and L. Walther, Ber., 63, 852 (1930).

3-METHYL-C^{14}-MORPHINE HYDROCHLORIDE (C_1^{14}-Codeine Hydrochloride)



F. N. H. Chang, J. F. Oneto, P. P. T. Sah, B. M. Tolbert and H. Rapoport, J. Org. Chem., 15, 634 (1950).

A. Procedure

To a frozen mixture (liquid nitrogen) of 6.0 g. (20 mmoles) of morphine *N*-oxide (Note 1) and sodium methoxide, prepared from 0.46 g. (20 mmoles) of sodium in 20 ml. of absolute methanol, is added 2.22 g. (15.6 mmoles)

of methyl-C^{14} iodide by vacuum distillation (Note 2). The mixture is refluxed on a steam-bath for 4 hours. Water (5 ml.) is added to the cool mixture, and sulfur dioxide is passed into the solution for 1 hour. Water (30 ml.) is added, and the methanol is distilled under reduced pressure. The residue is treated with 10 ml. of 6 *N* sodium hydroxide to dissolve the morphine, and the codeine is extracted with two 25-ml. portions and four 10-ml. portions of chloroform. The extract is washed with two 10-ml. portions of water, dried with potassium carbonate, filtered and evaporated to dryness. The codeine is dissolved in a minimum amount of benzene, and petroleum ether is added until no further yellowish-orange turbidity forms. The impurity is filtered off, excess petroleum ether is added to the filtrate, and the mixture is stored in a refrigerator to complete the precipitation of codeine. The solid is collected (m.p. 155°), and the mother liquor is reworked for a second crop. The codeine is dissolved in a small amount of absolute alcohol, and the solution is saturated with dry hydrogen chloride to precipitate the product. The mixture is evaporated to dryness on a steam-bath, and the product is recrystallized from 95% alcohol, collected, washed with cold absolute ethanol, and dried. The combined yield is 3.65 g. (62.8%), and the molar specific activity is unchanged from that of the starting material (Note 3). Analysis[1] by two-dimensional paper chromatography and radioautography shows the presence of only one radioactive compound.

B. Notes

1. In morphine itself the phenolic, tertiary amino, and allylic secondary alcohol groups are all capable of undergoing methylation with methyl iodide, all seven possible methylated morphine derivatives[2] being known. The amino group, being much more reactive than the alcoholic group, is temporarily blocked as the *N*-oxide. 3-Morphine methoxymethyl ether, prepared[2] in 63% yield by treating the sodium derivative of morphine with chloromethyl methyl ether, is oxidized with hydrogen peroxide[2] and precipitated as the crystalline acetone complex of methoxymethylmorphine *N*-oxide (73% yield) by the method of Faris and Small.[3] The desired morphine *N*-oxide is prepared[3,4,5] by hydrolysis of this (phenolic) ether in cold dilute sulfuric acid, followed by neutralization with ammonium hydroxide; yield 42.0% based on morphine, m.p. 271°.

2. The reaction flask is connected to a vacuum manifold through a condenser and stopcock.

3. The product has also been converted to the sulfate.

[1]A. A. Benson, J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Hass and W. Stepka, J. Am. Chem. Soc., 72, 1710 (1950).

[2]C. Mannich, Arch. Pharm., *254*, 349 (1916); J. Chem. Soc., *112*, (1), 473 (1917).

[3]B. F. Faris and L. Small, J. Org. Chem., *1*, 194 (1937).

[4]F. N. H. Chang, J. F. Oneto, P. P. T. Sah, B. M. Tolbert, and H. Rapoport, J. Org. Chem., *15*, 634 (1950).

[5]L. F. Small, U. S. 2,104,058; Chem. Abstracts, *32*, 1867 (1938).

NOR-*C*-CURARIN I METHOCHLORIDE-C^{14}
(C_1^{14}-*C*-Curarine I Chloride)

$$C_{19}H_{18}N_2 \xrightarrow{C^*H_3I} C_{20}H_{21}N_2I \cdot H_2O \xrightarrow{AgCl} C_{20}H_{21}N_2Cl$$

H. Schmid, K. Schmid, P. Waser and A. Ebnother, Helv. Chim. Acta, *34*, 2042 (1951).

A. Procedure (Note 1)

To a mixture of 0.02955 g. of crystallized nor-*C*-curarin I (Note 2) and 0.5 ml. of anhydrous benzene, frozen in liquid nitrogen, is added 0.121 g. of methyl-C^{14} iodide by vacuum distillation (10 μ). The reaction tube is closed, protected from light, and shaken, and the mixture is set aside for 14 hours at 20°, then heated for 2 hours at 60°. The solvent and excess methyl iodide are removed by vacuum distillation into an ampoule. The C_1^{14}-*C*-curarine I iodide, obtained in quantitative yield (Note 3), is dissolved in 5 ml. of pure acetone and 0.5 ml. of methanol, and the solution is shaken for 8 hours with 5 ml. of an aqueous suspension of silver chloride freshly prepared from 0.190 g. of silver nitrate. The insoluble halides are filtered off and washed with dilute methanol. The filtrate is evaporated at 40° under reduced pressure, and the residue is twice taken to dryness from absolute alcohol. The colorless varnish (0.03875 g.) is crystallized as shining, colorless needles from absolute methanol-ether (Note 4); yield 0.03804 g. (93%). The purity is checked by paper chromatography and radioautography (Note 5).

B. Notes

1. The structure of these compounds is not fully known. The labeled methyl group is attached to the basic N (b) atom. The preparation is adapted from the method of Karrer and Schmid.[1]

2. The starting material is prepared[1] by splitting methyl chloride from *C*-curarin I chloride by heating under high vacuum.

3. The product remains a monohydrate after drying for 8 hours over phosphorus pentoxide at 100°. The picrate melts at 309° (dec).

4. The product crystallizes as a tetrahydrate from aqueous solvent.

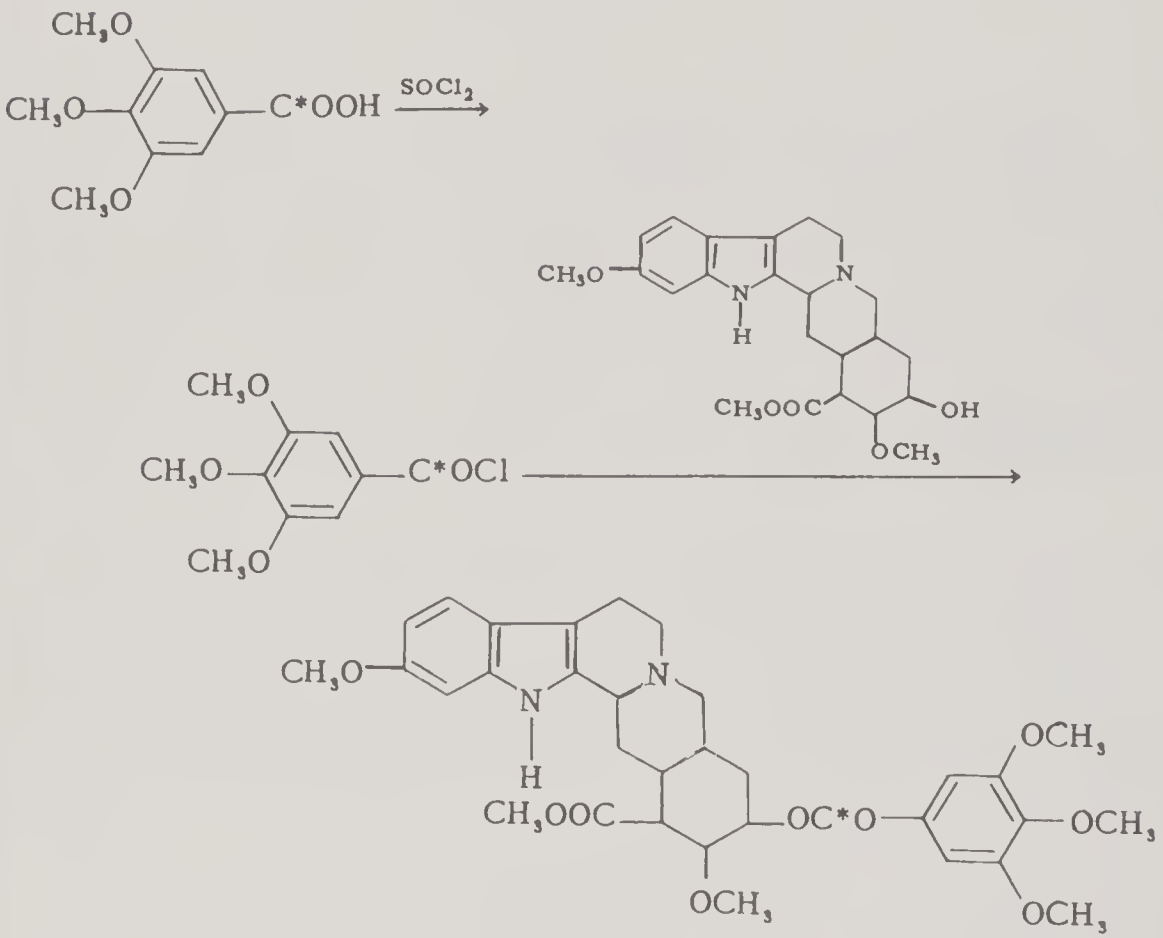
5. The solvent system used is ethyl acetate-pyridine-water.

[1]P. Karrer and H. Schmid, Helv. Chim. Acta, *29*, 1853 (1946).

METHYL 1,2,3,4,4a,5,7,8,13,13b,14,14a-DODECAHYDRO-2,11-DIMETHOXY-3-(3,4,5-TRIMETHOXYBENZOYLOXY-C^{14})-BENZ[*g*]INDOLO[2,3-*a*]QUINOLIZINE-1-CARBOXYLATE

[Methyl *O*-(3,4,5-Trimethoxybenzoyl-C^{14})reserpate]

(C_1^{14}-Reserpine)



P. Numerof, M. Gordon and J. M. Kelly, J. Pharmacol. Exp. Therap., *115*, 427 (1955).

A. Procedure (Note 1)

A solution of 0.030 g. (0.142 mmole) of 3,4,5-trimethoxybenzoic-C^{14} acid and 2 ml. of distilled thionyl chloride is stirred at room temperature for 1 hour, then heated to boiling, and the excess thionyl chloride is distilled (20 mm.). The residual 3,4,5-trimethoxybenzoyl-C^{14} chloride is evacuated to 0.2 mm. for 5 minutes, then treated with a solution of 0.0236 g. (0.057 mmole) of methyl reserpate[1] in 2 ml. of anhydrous pyri-

dine. The solution is stored overnight, diluted with 3 ml. of water, stored for 15 minutes and treated with 5 ml. of 3% sodium bicarbonate. The solution is extracted with four 10-ml. portions of chloroform, and the extract is dried over sodium sulfate and evaporated. The residue (0.047 g.) is dissolved in 10 ml. of benzene and chromatographed[2] on acid-washed alumina (pH 4.5). The major fractions are combined, evaporated and recrystallized from methanol; yield 0.0068 g. The m.p. (264-265° dec.) and ultraviolet spectrum are identical with those of an authentic sample.[1,2]

B. Notes

1. The procedure is an adaptation of the method of Furlenmeier.[1]

2. Reserpic acid (m.p. 239-245°), prepared by alkaline hydrolysis of *Rauwolfia serpentina* and isolated as the hydrochloride which is decomposed with silver carbonate, is esterified with diazomethane. Methyl reserpate melts at 235-239.5°, $[\alpha]_D$-101 ± 3° ($CHCl_3$); the ultraviolet spectrum is described.[1]

[1]A. Furlenmeier, R. Lucas, H. B. MacPhillamy, J. M. Miller and E. Schlittler, Experientia, 9, 331 (1953).

[2]N. Neuss, H. E. Boaz and J. W. Forbes, J. Am. Chem. Soc., 76, 2463 (1954).

CHAPTER 5

CARBONIC ACID DERIVATIVES

CARBON-C^{14}

METHOD I

$$C^*O_2 \xrightarrow{Mg} C^*$$

R. Abrams, J. Am. Chem. Soc., *71*, 3835 (1949).

A. Procedure (Note 1)

The dry carbon-C^{14} dioxide from 1.1 mmoles of barium carbonate-C^{14} is admitted to a quartz tube containing 2.5 mmoles of powdered magnesium in a thin-walled iron thimble. The thimble is heated to red heat, by intermittent heating of the tube with an oxygen flame, until the pressure drops to a constant value. The contents of the thimble are washed with *N* hydrochloric acid and water (Note 2) and dried; yield 0.76 mmole (89% purity).

METHOD II

$$CaC^*O_3 \xrightarrow{Li} C^*$$

I. W. Ruderman and C. S. Wu, Atomic Energy Commission Report, AECD-2412; Nuc. Sci. Abstracts, *2*, 74 (1949).

A. Procedure (Note 3)

A mixture of 0.00451 g. of barium carbonate-C^{14} and 0.4 g. of lithium is sealed under helium in a nickel tube (Note 4) and heated for 2 hours in a furnace at 650°. The cooled tube is cut open, and the contents are washed into a platinum dish with alcohol and water. The mixture is acidified with 6 *N* hydrochloric acid, and the supernatant solution is removed with a porous (superfine) stainless steel immersion tube by washing with

water. The tube is placed in a beaker of water, and air is passed through the tube to free any particles adhering to the disc. The combined carbon suspension is evaporated to dryness, then treated with concentrated sulfuric acid, and the mixture is taken to dryness on a hot plate. The residue is taken up in water, collected on a circle of filter paper placed in a fritted glass funnel (Note 5), washed with water and dried; radiochemical yield 60% (Note 6).

B. Notes

1. The procedure is adapted from the method described by Mellor.[1]

2. Recovered from the reaction mixture are: 5% unreacted carbon-C^{14} dioxide, 10% carbon-C^{14} monoxide and 15% acetylene-C_2^{14}.

3. The reaction of barium carbonate with lithium, sodium, potassium and magnesium at elevated temperatures was studied briefly. Reactors of Pyrex, quartz or copper were found to be unsatisfactory.

4. A 6-inch length of nickel tubing (1/2 in. O.D., wall thickness 3/64 in.) is flattened at one end in a vise and welded in an oxygen-gas flame. The other end is fitted with a rubber connection to a glass stopcock and a vacuum manifold having a helium inlet. The end of the charged tube adjacent to the rubber hose is flattened in a vise, bent over, flattened and welded shut, while the lower end is cooled in ice.

5. Collecting the product on fritted glass may result in loss of carbon through strong surface adsorption.

6. A large-scale preparation, with 5.05 g. of barium carbonate and 1.2 g. of lithium, gave a yield of 55% (purity 96%).

C. Other Preparations

The preparation of carbon-C^{14} black by the reduction of carbon-C^{14} dioxide with manganese[2] above 500° has been reported by Osipow.[3]

The preparation of carbon-C^{14} black (Philblack O) by several methods, all giving the same particle size, has been reported by Kirschenbaum:[4] 1.) carbon-C^{14} dioxide was heated in a quartz tube at 900–1000° for 5.5 hours with outgassed Philblack O, and the carbon monoxide formed was then pumped off, 2.) Philblack O was impregnated with C^{14}-glycine which was then decomposed.

Active carbon-C^{14} catalyst for the cracking of straight-chain hydrocarbons has been prepared[5] by treating active carbon with carbon-C^{14} dioxide at high temperatures.

[1]Mellor, *A Comprehensive Treatise on Inorganic and Theoretical Chemistry*, Longmans, London, 1925, Vol. 6, p. 71.

[2]A. N. Campbell and E. A. Brown, J. Am. Chem. Socl, *60*, 3055 (1938).

[3]L. Osipow, G. Segura, Jr., C. T. Snell and F. D. Snell, Ind. Eng. Chem., *45*, 2779 (1953).

[4]A. D. Kirschenbaum, C. W. Hoffman and A .V. Grosse, Anal. Chem., *23*, 1440 (1951).

[5]A. V. Grosse and E. Nodiff, 12th Intern. Congr. Pure and Appl. Chem., New York, *1951*, Abst. of Papers, 175.

POTASSIUM CYANIDE-C^{14}

METHOD I

$$BaC^*O_3 \xrightarrow[K]{NH_4Cl} KC^*N$$

F. L. J. Sixma, H. Hendriks, K. Helle, U. Hollstein and R. van Ling, Rec. trav. chim., *73*, 161 (1954).

A. Procedure

To a mixture of 0.200 g. of barium carbonate-C^{14} and 0.100 g. of ammonium chloride, under nitrogen in a Supremax tube (Note 1), is added 1–2 g. of potassium, which has been cleaned under absolute ether (Note 2). The ether is removed by evacuation and melting of the metal. The tube is closed, the potassium again is melted, and the contents of the tube are thoroughly mixed by shaking. The tube is placed in a furnace at 640° and heated for 60 minutes, after the 9 minutes required to bring the tube to temperature (Note 3). The tube is cooled and opened, and the contents are treated with alcohol to destroy the excess potassium. The mixture is transferred with hot water into a distilling flask and acidified with sulfuric acid; then the product is distilled, 15 ml. of distillate being collected under excess sodium hydroxide solution (Note 4). The yield is 94 ± 1.5% (average of runs), determined by titration with silver nitrate corrected for a blank of 4.7 ± 0.4% (average of 11 runs).

METHOD II

$$BaC^*O_3 \xrightarrow[Zn,\,Na]{NH_3} NaC^*N$$

J. K. Jeanes, Science, *118*, 719 (1953).

A. Procedure (Note 5)

A mixture of 1 mmole of powdered dry barium carbonate-C^{14}, 1.0 g. of zinc dust and 0.200 g. of sodium in pea-size chunks, contained in a por-

celain combustion boat, is placed in a Vycor combustion tube (19 × 600 mm.) in an atmosphere of anhydrous ammonia. Immediately beyond the boat, in the center of the tube, is a ball of iron wire (5.0 g., Note 6). The far end of the tube is attached to a bubble counter and a cylinder of anhydrous ammonia. While ammonia is passed through the tube at the rate of 3 bubbles per second, the portion of the tube containing the iron and reaction mixture is heated to and maintained at 650° for 4 hours; the gas flow is continued until the tube has cooled. The tube contents, except for the iron, are washed into a 250-ml. flask equipped with Kjeldahl distillation head. The solution is acidified with 2 *N* sulfuric acid, and 20–30 ml. of distillate is collected in a flask containing a 20% excess of *N* sodium or potassium hydroxide. The resulting solution may be used directly, or evaporated to dryness under vacuum. The yield, determined by the usual titration with silver nitrate, is quantitative, and the molar specific activity is unchanged from that of the starting material (Note 7).

METHOD III

$$BaC^*O_3 \xrightarrow[\substack{K \\ (NaOCH_3)}]{KN_3} NaC^*N$$

V. I. Maimind, B. V. Tokarev and M. M. Shemyakin, Doklady Akad. Nauk. S. S. S. R., *81*, 195 (1951); through Chem. Abstracts, 46, 3889 (1952).

A. Procedure (Note 8)

To an intimate mixture of finely divided barium carbonate-C^{14} (0.100–0.400 g.) and three parts of pure potassium azide, in a large Pyrex test tube under nitrogen, is added 1.0–1.2 g. of potassium (Note 9). The tube is tilted at a 45° angle and, in a slow nitrogen stream, is heated with a burner, then with an electric heater at 300–400° for 3–5 minutes. The mixture is next heated in a tube furnace at 750–780° for 2–3 minutes, then cooled and treated with 3–4 ml. of water, or 50% methanol. The resulting solution is acidified with 5 ml. of 40% sulfuric acid, and the hydrogen cyanide-C^{14} is distilled into *N* sodium methoxide in methanol yield 85–90% (Note 10).

METHOD IV

$$C^*O_2 \xrightarrow[NH_3]{K} KC^*N$$

J. A. Bos, Experientia, 7, 258 (1951).

A. Procedure (Note 11)

Approximately 1.4 g. of potassium under petroleum ether is weighed into a small flask sealed to a Pyrex tube (8 × 300 mm.) filled with nitrogen. The solvent is evaporated under vacuum, and the metal is distilled into the tube at 20μ. The tube is cooled, filled with nitrogen and sealed off from the flask. The potassium is dissolved by half-filling the tube with liquid ammonia. Evaporation of the solvent, while the tube is rotated, deposits the potassium in a thin film over most of the tube. Before the last of the ammonia is removed, the tube is reconnected to the vacuum manifold, and 1 mmole of carbon-C^{14} dioxide and 2 mmoles of dry ammonia are added by distillation. The sealed tube is heated in a bomb furnace to 620° in 8 minutes and held at that temperature for 12 minutes, then cooled in liquid nitrogen and opened (Note 12). The excess metal and hydrides are decomposed with ethanol and water, and the mixture is evaporated under vacuum. Hydrogen cyanide-C^{14} is released by acidification with formic acid and distilled with about 1 ml. of water into a trap containing excess alkali; yield 89.2–92.7%, determined by titration with silver nitrate.

METHOD V

$$C_6H_5CH_2C^*OOH \xrightarrow[\text{Cat.}]{NH_3} C_6H_5CH_2C^*N \xrightarrow[C_2H_5OH,\ Na]{[H]} NaC^*N$$

(a) (b)

W. R. Vaughan and D. I. McCane, J. Am. Chem. Soc., 76, 2504 (1954).

A. Procedure

(a) *Phenylacetonitrile-1-C^{14}* (Note 13). Phenylacetic-1-C^{14} acid (m.p. 76.5–78°, 70 g., 515 mmoles) is passed slowly through a heated dropping funnel (Note 14) during 5 hours into a metered stream of anhydrous ammonia (100 ml. per minute). The mixture is passed over preconditioned silica gel at 490° (Note 15). The effluent gases are passed through a condenser cooled with chilled water, then into a receiver cooled with an ice-salt bath. The crude product is separated from the aqueous layer (Note 16) and used without further purification; yield 55.3 g., 92% (Note 17).

(b) *Potassium Cyanide-C^{14}* (Note 18). To a refluxing, vigorously stirred mixture of 300 ml. of toluene and 65 g. (2.8 moles) of finely divided sodium is added a solution of 55.3 g. (0.472 mole) of crude phenylacetonitrile-1-C^{14} in 65 g. of absolute ethanol during 45 minutes (Note 19).

Absolute alcohol (65 g.) is added during 20 minutes; then the mixture is cooled and diluted cautiously with 200 ml. of water. The toluene layer is separated and extracted with three 100-ml. portions of water (Note 20). The combined aqueous solution is added to 400 ml. of 18 *N* sulfuric acid, and the evolved hydrogen cyanide-C^{14} is trapped in a solution of 35 g. of potassium hydroxide in 250 ml. of water, while 100 ml. of distillate is collected during 2 hours. The alkaline solution is evaporated to dryness, giving potassium cyanide-C^{14} in 81.2% yield based on crude nitrile (Note 21).

METHOD VI

$$(C_6H_5)_3CNa \xrightarrow{C^*O_2} \underset{(a)}{(C_6H_5)_3CC^*OOH} \xrightarrow{SOCl_2} (C_6H_5)_3CC^*OCl \xrightarrow{NH_4OH}$$

$$\underset{(b)}{(C_6H_5)_3CC^*ONH_2} \xrightarrow{P_2O_5} \underset{(c)}{(C_6H_5)_3CC^*N} \xrightarrow[C_2H_5OH]{Na} \underset{(d)}{NaC^*N}$$

B. Belleau and R. D. H. Heard, J. Am. Chem. Soc., *72*, 4268 (1950).

A. Procedure (Note 22)

(a) *Triphenylacetic-1-C^{14} Acid* (Note 23). To the dry carbon-C^{14} dioxide, liberated from 0.600 g. of barium carbonate-C^{14} and distilled into a 100-ml. reaction flask fitted with a dropping funnel, is added rapidly 30 ml. of 0.1 *N* triphenylmethylsodium (1.2 molar proportion) in ether,[1] after removal of the liquid nitrogen bath. The mixture is warmed to room temperature, and the flask is shaken vigorously for 2 minutes. An equal volume of 10% ammonium hydroxide is added, and the ether phase is separated and extracted with two portions of dilute ammonia. The combined alkaline extracts are washed with ether and acidified to precipitate the product; yield 0.780–0.800 g. (90–92%), m.p. 267°.

(b) *2,2,2-Triphenylacetamide-1-C^{14}* (Note 24). A mixture of 0.800 g. of triphenylacetic-1-C^{14} acid and 5 ml. of purified thionyl chloride is refluxed for 2.5 hours, then evaporated to dryness under vacuum. The triphenylacetyl-1-C^{14} chloride is pulverized under 10 ml. of concentrated ammonium hydroxide and set aside for 30 minutes. The product is collected and dried; yield 0.780 g. (95%), m.p. 246–248°.

(c) *Triphenylacetonitrile-1-C^{14}*. A mixture of 0.780 g. of 2,2,2-triphenylacetamide-1-C^{14}, 15 ml. of dry toluene and 5 g. of phosphorous pentoxide is refluxed for 50 hours. Water is added, and the aqueous layer is separated and extracted with four portions of benzene. The combined extracts are dried over potassium hydroxide, and the solvents are distilled; crude yield 0.730 g. (97%), m.p. 115–125°. Recrystallization from dilute acetone raises the melting point to 127–128°.

(d) *Sodium Cyanide-C^{14}.* To a solution of 0.730 g. of crude triphenylacetonitrile-1-C^{14} and 9.5 ml. of absolute ethanol, heated under reflux in a 50-ml. flask fitted with a sealed-in gas inlet (closed), is added 1 g. of sodium (in ten portions) during 30–40 minutes. The effluent gas, meanwhile, is passed through a trap containing sodium hydroxide solution, which is added to the reaction mixture at the end of the heating period (Note 25). The flask is fitted with a pressure-equalizing dropping funnel, a still head and a condenser with an outlet tube dipping below the surface of a 20% excess of *N* sodium hydroxide solution in the receiver. While the apparatus is swept with a gentle stream of nitrogen, the mixture is acidified by the dropwise addition of excess dilute sulfuric acid, then 15 ml. of distillate is collected. After removal of a slight turbidity by washing the distillate with ether, the solution may be used directly or evaporated to dryness under vacuum at room temperature; yield 90% (Note 26).

B. Notes

1. The tube is 1.6 × 17 cm. The etching of tubes is less with Supremax than with Pyrex glass, but the blank appears to be higher.

2. The yield drops to 50% when sodium is used. These investigators have made a comparative study of the available preparative methods for synthesizing isotopic cyanide.

3. The yield is 82% and 70% after 45 and 15 minutes of heating, respectively.

4. The recovery of hydrogen cyanide by distillation is 98.9 ± 0.6%. Prelog[2] has acidified with 2 *N* sulfuric acid and distilled the product into 3 ml. of 1 *N* carbonate-free potassium hydroxide. The distillate was titrated with 0.1 *N* silver nitrate and a slight excess was added, after the addition of 2.5 g. of sodium bicarbonate. The precipitated silver cyanide-C^{14} was centrifuged, washed with water and acetone and dried under vacuum at 80°; yield 0.1057 g. (78.0%). The reconversion to potassium cyanide-C^{14} is described, see decanedioic-1,10-C_2^{14} acid.

5. The procedure is a modification of the isotopic method of McCarter,[3] which achieved yields of 88–93% by employing the potassium salt. Hers[4] has employed the method of McCarter and converted the product to sodium cyanide-C^{14} by distillation of the regenerated hydrogen cyanide-C^{14} into sodium hydroxide. Nystrom[4a] has described the preparation by a modification of the method of McCarter,[3] heating 5 g. of zinc and 5 mmoles of potassium carbonate-C^{14} for 10 hours at 650° in a stream of ammonia; yield over 90%. Speer[5] has reported yields of 85–95%, and Block[6] 90% by the original procedure. Lemmon[7] has described a preparation at the 5-mmole scale (87–93% yield), essentially by the method of McCarter, but with 10–12 hours of heating instead of 4. The specific activity is al-

ways within 98% of agreement with the titration values. Wacker[8] has reported a yield of 95% by the described procedure. The method has been described by Freudenberg.[8a]

6. Hot iron catalyzes the thermal decomposition of ammonia to give some form of active nitrogen. An equal weight of iron powder, dispersed through a plug of Pyrex-glass wool, may be used.

7. Diaper[9] has reported that special precautions are necessary to prevent loss of cyanide by alkaline hydrolysis and volatilization of hydrogen cyanide during evaporation of aqueous solutions. The excess potassium hydroxide is neutralized with nonisotopic hydrogen cyanide, the mixture is basified with ammonia and concentrated under vacuum over calcium chloride. The presence of the anion of a mineral acid in the ammoniacal solution reduces the pH by buffering and results in the loss of considerable cyanide.

8. The method is a modification of the original method of Adamson,[10] by which barium carbonate-C^{14} and sodium azide in a slow stream of nitrogen were heated together in a test-tube, giving yields of 75–80%. As the original method has given erratic results in the hands of many workers,[7,11,12,13] there have been several modifications introduced. Henneberry[11] has obtained yields of 79.4–84.9%, and corrected the difficulty with explosions, by introducing a precise time-temperature control. MacDiarmid[14] has obtained consistent yields of over 70% by slowly adding a mixture of the powdered reactants to a hot test tube in a system designed to trap the products blown over by the many minute explosions. The method has been used by Wilson[15] and Goodenow.[16] Van den Bos[17] has obtained the same yield and overcome the explosions by employing sodium chloride as a diluent. The original method of Adamson has been used by Haley[18] and Reid[19] (yield 60%), and a modification has been described in detail by Carrick[20] (yield 75%).

9. Claus[12] has found that yield depends on particle size and intimacy of mixing of reactants, precipitation of the carbonate and azide under specific conditions giving a mixture of reproducible behavior. A small amount of carbide usually is formed.

10. Rothstein[21] has obtained a yield of 86.8% by a variation of this procedure employing sodium metal. Claus[12] has reported yields of 89–95% by employing sodium, controlling the azide decomposition at 275–315° by regulating the bath temperature, then heating at 900° for 10 minutes. Sixma (see Method I) has reported yields of 92.1 ± 0.7% (average of 6) by a slight modification in the temperature control of the described method.

11. Cramer and Kistiakowksy[22] have obtained potassium cyanide-C^{11} in yields of 50–60% by heating a vacuum-distilled mixture of 1 mmole of carbon-C^{11} dioxide, 1.0 g. of potassium and 2 mmoles of ammonia for 10

minutes at 525° in a sealed tube. Loftfield[23] has raised the average yield of cyanide-C^{14} to 92% by distributing the distilled metal as a mirror and by raising the temperature to 620°. Olynyk[24] has obtained yields of 61, 70 and 75% (at the 1 to 5.9-mmole scale) by essentially this latter procedure, with recovery of about 18% unreacted starting material. Borsook[25] has reported a 90% yield, and Selff and Tolbert[26] have described preparations with 80–90% yields at the 1–2-mmole level without difficulty. The described procedure is a slight modification of the procedure of Loftfield.

12. There is usually a small residual pressure.

13. The procedure is that of Mitchell.[27] The apparatus consists of a horizontal tube furnace,[5] automatically regulated to about 1° and containing a 23 x 560-mm. tube packed with 100 g. of commercial silica gel (6–16 mesh, Grade A).

14. The flow is regulated by means of a glass rod ground in the capillary tip.

15. The catalyst is conditioned by passing a slow stream of dry air over it at 220° for 4–5 hours, then slowly heating to operating temperature.

16. The nitrile is salted out by ammonium acetate and ammonium carbonate. The lower layer contains some product in the case of soluble nitriles.

17. An analytical procedure for determining the nitrile, which is difficult to separate from ammonia, is described by Mitchell.[27] Phenylacetonitrile distills at 119.4° (20 mm.).

18. The procedure is a modification of that described by Walter[28] for the reduction of cyanides.

19. The addition is just rapid enough to maintain vigorous refluxing.

20. The yield of sodium cyanide-C^{14} is 67–85% based on barium carbonate. This solution is suitable for the preparation of cuprous cyanide-C^{14}. The toluene layer may be extracted with dilute hydrochloric acid for recovery of about 10%[28] benzylamine-α-C^{14}.

21. The yield is over 90% when pure nitrile is used. The solution is assayed by a Volhard titration of an aliquot with 0.1 *N* silver nitrate. The entire synthesis may be scaled up or down satisfactorily.

22. The purification of intermediates is not necessary, and the method is equally adaptable to a micro- or macro-scale. Use of the method has been reported by Schayer.[29]

23. The procedure is adapted from the method of Schlenk and Marcus.[30] It is essential that oxygen be excluded from the reagents.

24. The method is that of Schmidlin and Hodgson.[13] Other dehydrating agents are less satisfactory: oxalyl chloride fails to effect dehydration, phosphorus pentachloride gives a lower yield, and thionyl chloride gives

an excellent yield of a product contaminated with a sulfur-containing impurity, which remains in the final cyanide.

25. Triphenylmethane precipitates.

26. An aliquot is titrated with silver nitrate. The over-all yield is 68–72% based on barium carbonate. Cyanide-C^{14} has been assayed as the silver salt by Adamson[10], and as the stable cobalticyanide complex by Baker.[31]

C. Other Preparations

Sodium cyanide-C^{14} has been prepared in 60–70% yield at the 1-mmole level by Abrams.[32] Carbon-C^{14} dioxide was reduced with magnesium at red heat, forming amorphous carbon-C^{14} (89% purity), which then was converted quantitatively to cyanide by heating with ammonia at 1000–1100° for 2.5 hours.

Sodium cyanide-C^{14} has been prepared by Spyker and Neish[33] in 85–93% yield, by heating sodium formate-C^{14} with 2 moles of sodamide for 12 minutes at 620° in a stainless steel bomb.

The purification of potassium cyanide-C^{13} (containing carbonate and hydroxide) by conversion to sodium cyanide-C^{13} has been described by Heyns[34] with the aid of an apparatus diagram. Hydrogen cyanide-C^{13}, liberated with use of 18 *N* sulfuric acid (60°), was dried over phosphorus pentoxide in a nitrogen stream, condensed in a spiral trap cooled with Dry Ice, absorbed in methanolic potassium hydroxide and assayed by titration of an aliquot with silver nitrate. The excess hydrogen cyanide was titrated with methanolic sodium hydroxide; yield 85%.

Triphenylacetic-1-C^{14} acid has been prepared by Pichat[35] in 89% yield, m.p. 268° (dec.), through carbonation of triphenylmagnesium chloride.

Triphenylacetyl-1-C^{14} chloride has been prepared in the described manner, by Pichat[35] (yield 86%), m.p. 128° after recrystallization from petroleum ether (b.p. 30–60°).

[1]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 607.

[2]V. Prelog, H. J. Urech, A. A. Bothner-By and J. Würsch, Helv. Chim. Acta, *38*, 1095 (1955).

[3]J. A. McCarter, J. Am. Chem. Soc., *73*, 483 (1951).

[4]H. G. Hers, J. Edelman and V. Ginsburg, J. Am. Chem. Soc., *76*, 5160 (1954).

[4a]A. L. Promislow, Univ. Microfilms (Ann Arbor, Mich.), Publ. No. 11,527; Chem. Abstracts, *49*, 11334 (1955).

[5]R. J. Speer, M. L. Humphries and A. Roberts, J. Am. Chem. Soc., *74*, 2443 (1952).

[6]W. Block, Z. Naturforsch., *8b*, 440 (1953).

[7]R. M. Lemmon, Atomic Energy Commission Report, UCRL-2026; Nuc. Sci. Abstracts, *7*, 1656 (1953).

[8]A. Wacker, H. Grisebach, A. Trebst and F. Weygand, Angew, Chem., *66*, 326 (1954).

[8a]K. Freudenberg and W. Fuchs, Ber., *87*, 1824 (1954).
[9]D. G. M. Diaper, S. Kirkwood and L. Marion, Can. J. Chem., *29*, 964 (1951).
[10]A. W. Adamson, J. Am. Chem. Soc., *69*, 2564 (1947).
[11]G. O. Henneberry and B. E. Baker, Can. J. Research, *28B*, 345 (1950); G. O. Henneberry, W. F. Oliver and B. E. Baker, Can. J. Chem., *29*, 229 (1951).
[12]C. J. Claus, D. C. Camp, J. L. Morgenthau, Jr., P. Olynyk and R. W. Helmkamp, Abstracts, 121st Meeting Am. Chem. Soc., 1952, 51K.
[13]J. Schmidlin and H. H. Hodgson, Ber., *41*, 438 (1908).
[14]A. G. MacDiarmid and N. F. Hall, J. Am. Chem. Soc., *75*, 4850 (1953).
[15]D. G. Wilson, K. W. King and R. H. Burris, J. Biol. Chem., *208*, 863 (1954).
[16]E. L. Goodenow and C. S. Garner, J. Am. Chem. Soc., *77*, 5268 (1955).
[17]B. G. Van den Bos, A. H. W. Aten, Jr., Rec. trav. chim., *70*, 495 (1951).
[18]E. E. Haley and J. P. Lambooy, J. Am. Chem. Soc., *76*, 2926 (1954).
[19]J. C. Reid, Atomic Energy Commission Report, AECU-586; Nuc. Sci. Abstracts, *4*, 419 (1950).
[20]W. L. Carrick and A. Fry, J. Am. Chem. Soc., *77*, 4381 (1955).
[21]M. Rothstein and C. J. Claus, J. Am. Chem. Soc., *75*, 2981 (1953).
[22]R. D. Cramer and G. B. Kistiakowsky, J. Biol. Chem., *137*, 549 (1941).
[23]R. B. Loftfield, Nucleonics, *1* (3), 54 (1947); Science, *106*, 544 (1947).
[24]P. Olynyk, D. B. Camp, A. M. Griffith, S. Woislowski and R. W. Helmkamp, J. Org. Chem., *13*, 465 (1948).
[25]H. Borsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighley and P. H. Lowy, J. Biol. Chem., *176*, 1383 (1948).
[26]R. E. Selff and B. M. Tolbert, Atomic Energy Commission Report, UCRL-1299; Nuc. Sci. Abstracts, *5*, 4406 (1951).
[27]J. A. Mitchell and E. E. Reid, J. Am. Chem. Soc., *53*, 321 (1931).
[28]L. A. Walter and S. M. McElvain, J. Am. Chem. Soc., *56*, 1614 (1934).
[29]R. W. Schayer, R. L. Smiley and E. H. Kaplan, J. Biol. Chem., *198*, 545 (1952).
[30]W. Schlenk and E. Marcus, Ber., *47*, 1666 (1914).
[31]S. B. Baker, D. E. Douglas and A. E. Seath, Atomic Energy Commission Report, NP-3178; Nuc. Sci. Abstracts, *5*, 5158 (1951).
[32]R. Abrams, J. Am. Chem. Soc., *71*, 3835 (1949).
[33]J. W. Spyker and A. C. Neish, Can. J. Chem., *30*, 461 (1952).
[34]K. Heyns and R. Brockmann, Z. Naturforsch., *9b*, 21 (1954).
[35]L. Pichat and M. Audinot, Bull. soc. chim. France, *19*, 466 (1952).

CUPROUS CYANIDE-C^{14}

$$NaC^*N \xrightarrow[NaHSO_3]{CuSO_4} Cu_2(C^*N)_2$$

J. C. Reid and J. C. Weaver, Cancer Research, *11*, 188 (1951).

A. Procedure (Note 1)

The apparatus consists of a 40-ml. centrifuge tube fitted with a three-hole rubber stopper, a sealed stirrer, an addition funnel and a gas outlet connected to an absorber filled with alkaline permanganate to trap any

cyanogen formed. To a well stirred solution of 1.50 g. (6.00 mmoles) of cupric sulfate pentahydrate, 10 ml. of water and 3 drops of 12 *N* sulfuric acid is added dropwise 15 ml. of 0.5 *N* sodium hydroxide containing 3.85 mmoles of sodium cyanide-C^{14} and 1.00 g. (3.97 mmoles) of sodium sulfite heptahydrate (Note 2). The product forms and precipitates rapidly. After 15 minutes, a current of air is drawn through the apparatus to sweep any gaseous products into the absorber. The product is collected by centrifugation, washed with water and dried under vacuum; yield 0.350 g., 100% (Note 3).

B. Notes

1. The procedure is adapted from the method of Barber.[1] The reduction of cupric sulfate is achieved at the expense of bisulfite instead of cyanide, one-half of which would otherwise be lost as cyanogen.[2]

2. Just before use, sufficient 12 *N* sulfuric acid is added to the alkaline solution to discharge the color of phenolphthalein.

3. The yield is usually about 80%. Vaughan[3] has described a similar preparation at the 385-mmole level (yield 93%), and Dauben[4] has reported a yield of 79.6% at the 180-mmole level.

C. Other Preparations

Cuprous cyanide-C^{13} has been prepared by Anker,[5] by shaking equimolar amounts of solid cuprous chloride and sodium cyanide-C^{13} in aqueous solution. Cuprous cyanide-C^{14} has been prepared by Martin[6] (91% yield) from cuprous chloride and sodium cyanide-C^{14}.

[1]H. J. Barber, J. Chem. Soc., *1943*, 79.
[2]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 46
[3]W. R. Vaughan and D. I. McCane, J. Am. Chem. Soc., 76, 2504 (1954).
[4]W. G. Dauben, C. F. Hiskey and M. A. Muhs, J. Am. Chem. Soc., 74, 2082 (1952).
[5]H. S. Anker, J. Biol. Chem., *176*, 1337 (1948).
[6]L. Martin and S. B. Baker, Atomic Energy Commission Report, NP-3177; Nuc. Sci. Abstracts, *5*, 5157 (1951).

POTASSIUM CYANATE-C^{14}

METHOD I

$$KC^*N \xrightarrow[Cu(OH)_2]{KMnO_4} KC^*NO$$

E. E. Haley and J. P. Lambooy, J. Am. Chem. Soc., 76, 2926 (1954).

To a stirred mixture of 0.3284 g. of potassium cyanide-C^{14}, 0.650 g. of potassium hydroxide, 2.5 ml. of water and cupric hydroxide catalyst

(freshly prepared from 0.487 g. of crystalline copper sulfate) in a 50-ml. centrifuge cone is added gradually a solution of 0.675 g. of potassium permanganate in 12 ml. of water. The mixture is heated at 50° for 5 minutes, then cooled, and the excess oxidant is decomposed by the gradual addition of 1 ml. of 3% hydrogen peroxide. The precipitate is separated by centrifuging and washed with three portions of water. The combined filtered solution is partially neutralized by the gradual addition of 5.3 ml. of 0.085 *N* sulfuric acid (0.45 meq.), then concentrated under reduced pressure (Note 2).

METHOD II

$$2\,(NH_2)_2C^*O + K_2CO_3 \longrightarrow 2\,KC^*NO + CO_2 + H_2 + 2\,NH_3$$

$$KC^*NO + AgNO_3 \longrightarrow AgC^*NO + KNO_3$$

D. L. Williams and A. R. Ronzio, J. Am. Chem. Soc., *74*, 2407 (1952).

A. Procedure (Note 3)

An intimate mixture of 0.0720 g. (1.2 mmoles) of urea-C^{14} and 0.0995 g. (0.72 mmole) of finely powdered, anhydrous potassium carbonate is fused in a tall slender platinum crucible. The crucible is heated near the top with a microburner, the conducted heat being allowed to initiate the fusion, thereby minimizing the loss of urea-C^{14} by entrainment in the evolved gases. As the partially melted mixture resolidifies, the burner is moved slowly toward the bottom of the crucible until a clear, quiescent liquid forms (Note 4). The cooled melt is dissolved in 2 ml. of water and transferred into a 40-ml. centrifuge cone with four 1-ml. portions of water. The excess carbonate is precipitated, with a slight excess of 0.1 *M* barium nitrate, as the barium salt, which is separated by centrifuging, the supernatant liquid being transferred into a second centrifuge cone by filter stick. The combined filtrate and 4 ml. of aqueous wash liquor is treated with a slight excess of 10% silver nitrate solution and cooled to 2–4°. The salt is collected by centrifuging and washed with six 1-ml. portions of water; yield of dried silver cyanate-C^{14} 0.1328 g., 74% (Note 5).

The silver cyanate-C^{14} is converted readily to the ammonium salt by treatment with ammonium chloride in aqueous suspension.

B. Notes

1. The procedure is a modification of that of Gall.[1] The isotopic preparation after Gall[1] has been described in detail by Smith.[2]

2. The yield is quantitative. The solution is used directly for the preparation of urea-C^{14}, Method II.

3. The procedure is an adaptation of that of Scattergood.[3]

4. Prolonged heating of the fused cyanate gradually forms potassium carbonate.

5. A trial experiment with urea and potassium carbonate-C^{14} gave non-isotopic cyanate, thereby showing that the isotopic carbon of the product comes only from urea.

C. Other Preparations

The preparation of potassium cyanate-C^{14} by Method I has been reported by Weed.[4] The preparation by Method II has been described by Smith,[5] Mandel[6] (95% yield) and Liebermann.[7]

[1]H. Gall and G. Lehman, Ber., *61B*, 670 (1928); Chem. Abstracts, *22*, 2528 (1922).

[2]L. H. Smith, Jr. and P. Yates, J. Am. Chem. Soc., *76*, 6080 (1954).

[3]A. Scattergood, *Inorganic Syntheses*, Vol. II, McGraw, New York, 1946, p. 87.

[4]L. L. Weed and D. W. Wilson, J. Biol. Chem., *189*, 435 (1951).

[5]L. H. Smith, Jr., J. Am. Chem. Soc., *77*, 6691 (1955).

[6]H. G. Mandel and C. L. Brown, The George Washington University School of Medicine, private communication.

[7]I. Liebermann and A. Kornberg, J. Biol. Chem., *207*, 911 (1954); *212*, 909 (1955).

SODIUM THIOCYANATE-C^{14}

$$NaC^*N \xrightarrow{S} NaSC^*N$$

METHOD I

J. C. Reid, M. O. Landefeld and J. L. Simpson, J. Natl. Cancer Inst., *12*, 929 (1952).

A. Procedure

A mixture of sodium cyanide-C^{14}, a quantity of ethyl carbonate equivalent to the free alkali present (Note 1), and sulfur (20% excess) is refluxed in ethanol for 1 hour. The solution is saturated with carbon dioxide, filtered and evaporated to dryness; yield quantitative.

METHOD II

H. Borsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighley and P. H. Lowy, J. Biol. Chem., *187*, 839 (1950).

A. Procedure (Note 2)

A mixture of 0.056 g. of sodium cyanide-C^{14}, 0.040 g. of sulfur and 1.7 ml. of acetone is refluxed for 45 minutes. The solvent is drawn off,

the residue is mixed with 0.020 g. of sulfur and 1.5 ml. of acetone, and the mixture is refluxed for 45 minutes. The combined acetone solutions are evaporated with a stream of nitrogen, and the residue is dried over sulfuric acid; yield quantitative (Note 3).

METHOD III

D. B. Sprinson and D. Rittenberg, J. Biol. Chem., *198*, 658 (1952).

A. Procedure (Note 4)

A mixture of 0.035 g. of sodium cyanide-C^{14}, 1.0 g. of potassium cyanide (95% purity), 3.0 of sulfur and 0.08 g. of potassium hydroxide is heated at 310° for 15 minutes under nitrogen. The cooled mass is powdered and extracted with four 75-ml. portions of absolute alcohol, and the solution is evaporated under vacuum. The residue is redissolved in absolute alcohol and saturated with carbon dioxide, and the filtered solution is evaporated; yield 1.4 g.

B. Notes

1. Since free alkali gives rise to troublesome complex sulfur compounds, the ester is employed to reduce the basicity through saponification.

2. The procedure is an adaptation of the method of Castiglioni.[1]

3. The yield is based on the titration values from trial runs. The product contains some sulfur which may be removed by extraction of the product with water and by centrifugation.

4. The procedure is an adaptation of the method described by Vanino.[2]

C. Other Preparations

The preparation of sodium thiocyanate-C^{14} by Method II has been reported by Toporek[3] and described by Adamson.[4]

[1]A. Castiglioni, Gazz. chim. ital., *63*, 171 (1933); Chem. Abstracts, *27*, 3700 (1933).

[2]L. Vanino, *Handbuch der praparativen Chemie*, Stuttgart, *I*, 387 (1925).

[3]M. Toporek, L. L. Miller and W. F. Bale, J. Biol. Chem., *198*, 839 (1952).

[4]A. W. Adamson and R. G. Wilkins, J. Am. Chem. Soc., *76*, 3379 (1954).

CARBON-C* MONOXIDE

METHOD I

$$CaC^*O_3 \xrightarrow{Zn} C^*O + CaO + ZnO$$

S. Weinhouse, J. Am. Chem. Soc., *70*, 442 (1948).

A. Procedure

Carbon-C^{13} Monoxide. A mixture of 10 mmoles of calcium carbonate-C^{13} and 20 mmoles of zinc dust (Note 1) is heated at 700–750° in an evacuated quartz tube attached to an evacuated system of known volume. The rate and extent of gas evolution are measured conveniently with a manometer; yield 216 ml. N.P.T. of gas of 99.1% purity (Note 2). There is no isotopic dilution.

Larger-scale preparations (up to 5 l.) are made at atmospheric pressure by heating the reactants in a Pyrex tube and collecting the product by gas displacement over water or oil.

METHOD II

$$C^*O_2 \xrightarrow[\Delta]{Zn} C^*O + ZnO$$

H. L. Huston and T. H. Norris, J. Am. Chem. Soc., *70*, 1968 (1948).

A. Procedure

Carbon-C^{14} Monoxide. The carbon-C^{14} dioxide from 0.02 g. of barium carbonate-C^{14} is passed, alternately clockwise and counterclockwise, by means of a Toepler pump, through a tube containing 50 g. of zinc at 385 ± 5° in a high-vacuum system (Note 3). Unreacted carbon dioxide is frozen into an adjacent trap with liquid nitrogen, after each traverse through the zinc, while the carbon-C^{14} monoxide is pumped back into the carbon dioxide generator for storage and pressure measurement to determine the extent of reaction, see phosgene-C^{14}, Figure V, 2.

The first pass through the zinc produces about 50% conversion, and five or six more traverses make the yield quantitative within the 0.4% limit of error in reading the manometer (Note 4).

B. Notes

1. An excess of zinc dust is required, as some is sublimed at the temperature employed. Huston[1] recommends that the 20-mesh zinc be cleaned with dilute acetic acid, washed and dried to remove the zinc carbonate which otherwise leads to a small blank.

2. The impurities, determined by mass-spectrometric analysis, are 0.9% C^*O_2 and traces of H_2 and H_2O. There is no reaction with barium carbonate under these conditions.

3. Each batch of fresh cleaned zinc (see Note 1) is baked overnight at 370° under vacuum. The reduction is too slow at 370°, but above 400° the zinc tends to lose its porosity. Bernstein[2] has employed temperatures as high as 435°, but found it advantageous to support the

zinc dust on 5% asbestos fiber to give greater porosity; small pellets were made from the moistened mixture and dried at 110° for 24 hours. Stranks[3] states that magnesium compounds in asbestos tend to cause reduction to elementary carbon. Murray[4] has obtained yields of 95% at the 2.8-mmole level with use of an acid-washed asbestos and thermal diffusion as described by Bernstein,[2] see Figure V, 1.

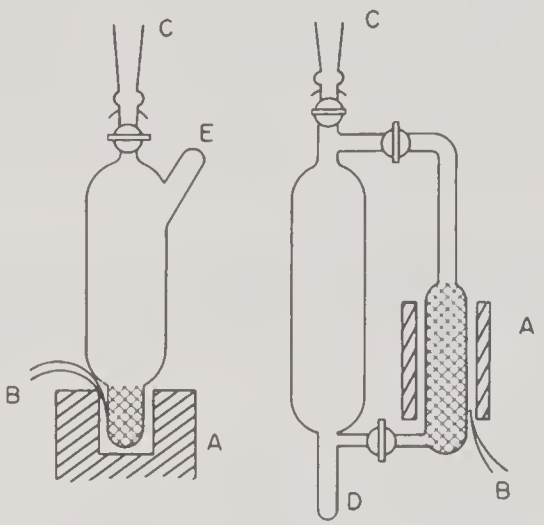


Fig. V,1. Semimicro and micro thermal convection systems for reducing carbon dioxide (R. B. Bernstein and T. I. Taylor), A, furnace; B, thermocouple; C, connection to vacuum manifold; D, freezing-tip; E, inlet for zinc-on-asbestos pellets.

4. Bernstein has reported that a single passage of carbon dioxide through zinc at 400° (at a rate of 100 cc. per minute) gives a 99.5% conversion. The carbon monoxide concentration was measured in an Orsat apparatus to ±0.2%.

Stranks[3] has used a temperature of 405 ± 2° (2 hours) for the isotopic preparation, and removed traces of carbon dioxide and oxygen (0.5%) with solid potassium hydroxide and sodium mirrors (cooled with liquid nitrogen), respectively.

Isotopic carbon monoxide is readily converted back to isotopic carbon dioxide by passage through copper oxide at 400°. Attree[5] has measured the isotope effect in combustion of carbon monoxide in oxygen on the walls of a vessel, the relative reaction rates being 1.00, 0.985 and 0.945 for C^{12}, C^{13}, C^{14}, respectively. There is less than 1% exchange between carbon-C^{14} monoxide and carbon dioxide at 620° during 1 hour.[5]

C. Other Preparations

Carbon-C^{13} monoxide has been prepared in 99% yield at the 0.2-millimole scale by Bernstein,[2] who modified Method II to the extent of employing a system (Figure V,1) relying upon thermal diffusion to pass the

gas mixture over the zinc pellets. Attree[5] has presented a diagram of a similar convection system employing glass beads covered with zinc dust at 400°.

Carbon-C^{14} monoxide has been prepared by Kummer[6] by exchange between carbon-C^{14} dioxide and carbon monoxide over a hot tungsten filament. The radiochemical yield is quantitative, but the specific activity is necessarily lowered by dilution.

The formation of carbon-C^{14} monoxide by the reduction of carbon-C^{14} dioxide with carbon at high temperature has been studied by Bonner.[7]

Carbon-C^{14} monoxide has been prepared by Melville[8] by dehydrating formic-C^{14} acid with sulfuric acid. Ropp[9] has used the method in a study of isotope effect and temperature.

[1]J. L. Huston and T. H. Norris, J. Am. Chem. Soc., *70*, 1968 (1948).
[2]R. B. Bernstein and T. I. Taylor, Science, *106*, 498 (1947).
[3]D. R. Stranks, J. Chem. Phys., *21*, 2232 (1953); Trans. Faraday Soc., *51*, 514 (1955).
[4]A. Murray III, unpublished work.
[5]R. W. Attree, F. Brown, G. E. Dunn and M. Lounsbury, Can. J. Chem., *32*, 921 (1954); F. Brown, Trans. Faraday Soc., *48*, 1005 (1952).
[6]J. T. Kummer, J. Am. Chem. Soc., *69*, 2239 (1947).
[7]F. Bonner and J. Turkevich, J. Am. Chem. Soc., *73*, 561 (1951).
[8]D. B. Melville, J. G. Pierce and C. W. H. Partridge, J. Biol. Chem., *180*, 299 (1949).
[9]G. A. Ropp, A. J. Weinberger and O. K. Neville, J. Am. Chem. Soc., *73*, 5573 (1951).

CARBON-C^{13} DIOXIDE

METHOD I

$$KC^*N \xrightarrow[NaOH]{KMnO_4} Na_2C^*O_3 \xrightarrow{H^+} C^*O_2$$

J. D. Cox, H. S. Turner and R. J. Warne, J. Chem. Soc., *1950*, 3167.

A. Procedure (Note 1)

To a solution of 50 mmoles of potassium cyanide-C^{13} in 25 ml. of carbonate-free water is added a 20% excess of 0.25 *N* barium hydroxide over that required to precipitate any carbonate (Note 2). The precipitate is filtered off and washed with 50 ml. of water. To the combined filtrate is added 16 g. of potassium permanganate, 45 ml. of carbonate-free 6 *N* sodium hydroxide and sufficient water to make the volume 200 ml. The solution is refluxed for 4 hours, while a slow stream of carbon dioxide-free nitrogen is bubbled through. The condenser is replaced by a drop-

ping funnel, and the cooled mixture is acidified by the dropwise addition of 35 ml. of 10 *N* sulfuric acid during 30 minutes, followed by 75 ml. added rapidly. The evolved carbon-C^{13} dioxide is passed in the nitrogen stream through a gas-washing bottle containing concentrated sulfuric acid and is collected in two traps at −198° (Note 3), complete evolution being ensured by heating the mixture at 50° for 2 hours. The liquid nitrogen-cooled traps are evacuated at a vacuum manifold (0.1 μ), and the product is expanded into a volume of 1.5 l., then condensed into a trap at −198° and dried by vacuum sublimation at the temperature of a Dry Ice-methanol bath.

METHOD II

$$KC^*N \xrightarrow{Zn^{++}} Zn(C^*N)_2 \xrightarrow[CuO]{O_2} C^*O_2 \xrightarrow{Ba(OH)_2} BaC^*O_3$$

J. Baddiley, G. Ehrensvärd, R. Johansson, L. Reio, E. Saluste and R. Stjernholm, J. Biol. Chem., *183*, 771 (1950).

A. Procedure

Potassium cyanide-C^{13} (81 g.) is precipitated as zinc cyanide-C^{13} (q.v.), which is dried, mixed with an equal weight of copper oxide and burned (2-g. portions) with oxygen in a quartz tube. The carbon-C^{13} dioxide is absorbed in barium hydroxide (Note 4), and the barium carbonate-C^{13} is centrifuged, washed and dried; yield 197 g. (80.4%).

In a similar manner, barium carbonate-C^{13} is formed[1,2] by burning methane-C^{13} (Note 5).

B. Notes

1. The isotopic procedure is essentially that described by Weinhouse,[3] and is satisfactory at the 3 to 220-mmole scale.

2. The commercial sample was a nonhomogeneous mixture of 78–90% potassium cyanide, 22–10% carbonate and less than 2% formate. An aliquot of a solution is assayed[4] for cyanide by titration with 0.02 *N* silver nitrate in the presence of ammonia and potassium iodide; formate is determined[3] by titration of an aliquot with 0.02 *N* permanganate, following displacement of hydrogen cyanide by bubbling carbon dioxide for 6 hours; the material difference is assumed to be carbonate.

3. A diagram of the apparatus is presented by Cox. The two traps are fitted with stopcocks and a guard tube containing magnesium perchlorate and soda lime.

4. A solution of 0.25 *N* barium hydroxide containing 2 g. of barium chloride dihydrate per 100 ml. has been recommended by Van Slyke[5] and

Anderson,[6] while Lindenbaum[7] has employed a saturated solution of barium hydroxide. Diagrams of suitable apparatus, as well as systems for wet and dry combustion of isotopic organic materials, are presented.[5-8] Wherever there is a possibility of isotope effect in the combustion of an organic compound, a homogeneous sample of barium carbonate-C^{14} may be obtained by precipitating the salt from an alkaline carbonate-C^{14} solution, rather than passing the gas directly into a solution containing barium ions.

5. Methane-C^{13} is forced through a microburner, fitted into a glass candle, and burned in a stream of oxygen. Safe uninterrupted burning is achieved by maintaining a continuous spark at the tip with a 5000-volt 60-cycle transformer. The carbon-C^{13} dioxide is absorbed in carbonate-free 10% sodium hydroxide solution and precipitated by addition of barium chloride solution; yield 85–95%.

[1]N. S. Olsen, A. Hemingway and A. O. Nier, J. Biol. Chem., *148*, 611 (1943).
[2]W. Sakami, W. E. Evans and S. Gurin, J. Am. Chem. Soc., *69*, 1110 (1947).
[3]S. Weinhouse, G. Medes and N. F. Floyd, J. Biol. Chem., *155*, 143 (1944).
[4]A. Vogel, *Quantitative Inorganic Analysis*, Longmans Green, London, 1939, pp. 327, 361.
[5]D. D. Van Slyke, R. Steele and J. Plazin, J. Biol. Chem., *192*, 769 (1951).
[6]R. C. Anderson, Y. Delabarre and A. A. Bothner-By, Anal. Chem., *24*, 1298 (1952).
[7]A. Lindenbaum, J. Schubert and W. D. Armstrong, Anal. Chem., *20*, 1120 (1948).
[8]R. F Glascock, *Isotopic Gas Analysis for Biochemists*, Academic Press, New York, 1944.

CARBON-C^{14} DIOXIDE

METHOD I

$$BaC^*O_3 \xrightarrow{H_2SO_4} C^*O_2$$

W. G. Dauben, J. C. Reid and P. E. Yankwich, Anal. Chem., *19*, 828 (1947); C. Heidelberger, P. Brewer and W. G. Dauben, J. Am. Chem. Soc., *69*, 1389 (1947).

A. Procedure (Note 1)

For a description of the procedure (Note 2) and vacuum apparatus, see benzoic-C^{14} acid; also see *p*-aminobenzoic-C^{14} acid.

METHOD II

$$BaC^*O_3 \xrightarrow[\triangle]{PbCl_2} [PbC^*O_3] \xrightarrow[\triangle]{} C^*O_2 + PbO$$

N. Zwiebel, J. Turkevich and W. W. Miller, J. Am. Chem. Soc., *71*, 376 (1949).

A. Procedure

To 0.005–0.200 g. of barium carbonate-C^{14} in a Pyrex test tube (Note 3) is added a layer of 5–10 times the weight of dry powdered lead chloride (Note 4) and a wad of glass wool. The tube is attached to a vacuum system and pumped on at 100°. The product is liberated by melting the mixture with a microburner (Note 5).

METHOD III

$$BaC^*O_3 \xrightarrow{HClO_4} C^*O_2$$

J. D. Cox, H. S. Turner and R. J. Warne, J. Chem. Soc., *1950*, 3167.

A. Procedure (Note 6)

The apparatus consists of a 100-ml. round-bottomed generator flask attached, through a pressure-compensating addition funnel, a plug of glass wool and a trap at −78°, to a vacuum manifold equipped with a trap at −196°. To a mixture of 3.90 g. of barium carbonate-C^{14} and 5 ml. of water, frozen at −78° under vacuum, is added 15 ml. of 60% perchloric acid. The freezing bath is removed, the mixture is warmed to 30°, the generator is closed off from the cold trap (−78°), and the product is dried by vacuum sublimation at −78° (Note 7). The yield is 100%, based on pressure measurements.

B. Notes

1. The various methods of releasing carbon dioxide from barium carbonate by acidification are about equivalent in yield, but give varying amounts of water and acidic impurities, depending upon the acid selected. The solubility of carbon dioxide at room temperature in sulfuric acid or dilute phosphoric, hydrochloric and perchloric acid solutions is not greatly different from that in water (0.8 cc./ml. at 1 atmosphere carbon dioxide pressure). Over hydrochloric acid at 25°, the vapor pressures (mm.) of water and hydrogen chloride, respectively, are: 2 *N*, 21.8, 0.0013; 6 *N*, 15.4, 0.148. The vapor pressure of perchloric acid in dilute aqueous solution is negligible.

Finely divided solid drying agents, such as phosphorus pentoxide, adsorb some carbon dioxide.

2. Similar procedures employing atmospheric pressure and a stream of nitrogen to transport the product have been reported by Harwood[1] and Lemmon.[2] The use of 85% phosphoric acid in this application has been reported by Huston[3] and Kummer;[4] diagrams of the apparatus are presented. Drying was achieved by passage of the gas through a tube filled with phosphorus pentoxide,[3] or a cold trap[4] at −78°, for the vapor pressure of water over 85% phosphoric acid at room temperature is 2.2 mm.

Barium phosphate has the disadvantage of being insoluble in phosphoric acid.

3. To prevent cracking upon cooling, the tube is of sufficient diameter that the final melt cannot fill a full diameter.

4. The fusion temperature (501°) may be lowered by using a 50–50 mixture of lead chloride-silver chloride.

5. Lead oxide decomposes at 750° with evolution of oxygen. Stevens[5] has effected the reaction by heating to about 350°, and Stranks[6] has used temperatures of 400–450° (4 hours at the 10-mmole level). Cox,[7] employing the method at the fractional-mmole level, has observed that most commercial lead chloride evolves some carbon dioxide on fusion.

6. The procedure is satisfactory at the 0.5 to 35-mmole level (Note 1). A similar procedure at the 0.5-mmole scale, employing 5 *N* perchloric acid and atmospheric pressure, is described by Collins;[8] a diagram of the apparatus is presented. The product is transported in a stream of nitrogen and dried by passage through a trap at −50°. Barium perchlorate is soluble.

7. Sakami[9] has employed 40% perchloric acid and dried the product by passage through Anhydrone, while Baddiley[10] has used 30% acid. A diagram of the apparatus is presented.[11]

C. Other Preparations

The generation of carbon-C^{14} dioxide by roasting barium carbonate-C^{14} at 1100° in a quartz tube has been reported by Inghram.[12] The yield is not quantitative on a large scale.

5 *N* Lactic acid has been employed by Buchanan,[13] 8 *N* hydrochloric acid by Spector[14] and 6 *N* hydrochloric acid by Leslie[15] to liberate isotopic carbon dioxide from the barium salt.

[1] H. J. Harwood and A. W. Ralston, J. Org. Chem., *12*, 740 (1947).

[2] M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 178.

[3] J. L. Huston and T. H. Norris, J. Am. Chem. Soc., *70*, 1968 (1948).

[4] J. T. Kummer, J. Am. Chem. Soc., 69, 2239 (1947).

[5] W. H. Stevens and R. W. Attree, Can. J. Research, *27B*, 807 (1949); R. C. Hawkins, R. F. Hunter, W. B. Mann and W. H. Stevens, Can. J. Research, *27B*, 545 (1949).

[6] D. R. Stranks, J. Chem. Phys., *21*, 2232 (1953); Trans. Faraday Soc., *51*, 514 (1955).

[7] J. D. Cox, H. S. Turner and R. J. Warne, J. Chem. Soc., *1950*, 3167.

[8] C. J. Collins, J. Am. Chem. Soc., *70*, 2418 (1948).

[9] W. Sakami, W. E. Evans and S. Gurin, J. Am. Chem. Soc., *69*, 1110 (1947).

[10] J. Baddiley, G. Ehrensvärd, R. Johansson, L. Reio, E. Saluste and R. Stjernholm, J. Biol. Chem., *183*, 771 (1950).

[11] M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 176; H. E. Skipper, C. E. Bryan, L. White, Jr., and O. S. Hutchison, J. Biol. Chem., *173*, 371 (1948).

[12]M. G. Inghram, Atomic Energy Commission Report, MDDC-60.
[13]J. M. Buchanan, A. B. Hastings and F. B. Nesbett, J. Biol. Chem., *150*, 413 (1943).
[14]L. B. Spector, Atomic Energy Commission Report, MDDC-532.
[15]W. B. Leslie, Atomic Energy Commission Report, MDDC-674.

DIAZOMETHANE-C*

METHOD I

$$(C^*H_3NH_2)_2 \cdot H_2SO_4 \xrightarrow[H_2SO_4]{NaCNO} C^*H_3NHCONH_2 \xrightarrow{HONO}$$

$$C^*H_3N(NO)CONH_2 \xrightarrow{KOH} C^*H_2N_2$$

J. D. Cox and R. J. Warne, J. Chem. Soc., *1951*, 1896.

A. Procedure (Note 1)

Diazomethane-C^{13}. To 3.10 mmoles of methylamine-C^{13} sulfate in 2 ml. of water is added 0.6 g. of sodium cyanate and a few drops of 0.1 *N* sulfuric acid; then the solution is refluxed for 15 minutes. The stirred mixture of methyl-C^{13}-urea (Note 2) is acidified at −5° with 3.5 ml. of 2.75 *N* sulfuric acid, then treated in the cold, portionwise during 1 hour, with 0.5 g. of sodium nitrite. Ether is added and the aqueous phase is extracted with three portions of cold ether (Note 3). The combined ether solution of 1-methyl-C^{13}-1-nitrosourea is added to a cooled 10 *N* sodium hydroxide solution (Note 4), and the resulting ethereal solution of diazomethane-C^{13} is distilled into a trap at −78° (Note 5). The yield is 3.56 mmoles (57%), determined by treating the solution with weighed portions of *meso*-dibromosuccinic acid (0.51 g., 1.78 meq.) until the yellow color is just discharged (Note 6). There is no isotopic dilution (Note 7).

METHOD II

$$C^*H_3NH_2 \cdot HCl \xrightarrow{(NH_2)_2CO} C^*H_3NHCONH_2 \xrightarrow{HONO}$$

$$C^*H_3N(NO)CONH_2 \xrightarrow{KOH} C^*H_2N_2$$

(a) (b)

R. D. H. Heard, J. R. Jamieson and S. Solomon, J. Am. Chem. Soc., *73*, 4985 (1951).

A. Procedure (Note 8)

(a) *1-Methyl-C^{14}-1-nitrosourea.* A solution of 3.55 mmoles of crude methylamine-C^{14} hydrochloride and 0.711 g. (11.85 mmoles) of urea in 1 ml. of water is refluxed for 3 hours. To the stirred, ice-cooled solution of methyl-C^{14}-urea is added 5.6 ml. of an aqueous solution containing 0.790 g. (10.7 mmoles) of carrier methylurea and 1.3 g. of sodium nitrite, followed by the dropwise addition over a period of 15 minutes of a solution of 0.65 ml. of sulfuric acid in 7.1 g. of ice water (Note 9). The precipitate is collected and dried under vacuum; yield 1.04 g. (55% based on cyanide), m.p. 120–123° (Note 10).

(b) *Diazomethane-C^{14}.* To a stirred ice-cooled mixture of 50 ml. of ether and 3 ml. of 50% potassium hydroxide solution is added 1.04 g. of 1-methyl-C^{14}-1-nitrosourea. The flask is fitted with a still-head, condenser, and an adapter dipping beneath the surface of ether in a receiver cooled in an ice-salt bath. The exit gases are passed through a second similar trap. The reaction mixture is heated in a water-bath at 50°, and most (but not all) of the ether is distilled; yield 0.319 g., 7.6 mmoles, 41–42% based on cyanide (Note 11). For radiochemical assay, 0.050 g. of 3-oxo-4-etiocholenic acid is esterified with a suitable aliquot of the ether solution (see deoxycorticosterone-3-C^{14} 21-acetate). The methyl-C^{14} ester, separated and purified by alumina chromatography, melts at 132–133°.

B. Notes

1. The procedure is a modification of the method of Arndt and Amende.[1] This method is more readily adaptable to a small-scale preparation[2] than are those involving the reaction of methylamine hydrochloride with urea[3] or mesityl oxide.[4]

2. Jones[2] has obtained methyl-C^{14}-urea quantitatively from methyl-C^{14}-amine hydrochloride in this manner by eluting the dried reaction mixture with hot methanol.

3. The yield[1] of 1-methyl-1-nitrosourea is 84–98% from 1.5 moles of methylamine hydrochloride. The isolated product must be kept below 20°; stability may be increased by addition of a drop of acetic acid. Jones[2] has obtained 1-methyl-C^{14}-1-nitrosourea in 77% yield based on sodium cyanide (76% radiochemical yield).

4. In a similar preparation the proportions employed were: 3 g. of 1-methyl-1-nitrosourea, 50 ml. of ether and 10 ml. of 10 *N* sodium hydroxide.

5. It is recommended[1] in the larger-scale preparations that not all the ether be distilled.

6. The yield at the 30-mmole level is 65–68%, or up to 55% based on barium carbonate-C^{13}. Jones[2] has reported a 60% yield of diazomethane-C^{14} based on sodium cyanide (radiochemical yield 54%), assayed as the methyl-C^{14} 3,5-dinitro-*o*-toluate.

7. The ethereal solution is washed with sodium bicarbonate solution and water, dried over sodium sulfate and evaporated. The residue is crystallized from petroleum ether to give prisms of bis(methyl-C^{13}) *meso*-dibromosuccinate, m.p. 63°, which is oxidized to carbon-C^{13} dioxide for assay.

8. The procedure is an adaptation of the method of Arndt.[5]

9. The usual order of nitrosation is reversed, without affecting the yield, in order to minimize loss.

10. The compound is reasonably stable in the cold, but decomposes badly in thin films at room temperature. The usual yield[5] for this reaction is 66–72%. Hershberg[6] has reported a yield of 11.9 g. of 1-methyl-C^{13}-1-nitrosourea from the crude methylamine-C^{13} hydrochloride prepared from 6.22 g. of sodium cyanide-C^{13}.

11. The usual yield[5] for this reaction is 63–70%, although Cox[7] has reported 91%. Raffauf[8] has reported a 57% yield of diazomethane-C^{14}, and Hershberg[6] has obtained 37.8 mmoles of diazomethane-C^{13} in 30% yield based on sodium cyanide. An aliquot of the ether solution may be assayed by esterification of excess benzoic acid and back-titration with alkali,[5] or by the excellent micromethod of Raffauf[8] (see C_1^{14}-colchicine). Water may be removed from the preparation by drying the ether solution over pellets of potassium hydroxide, and then over sodium wire.

[1] F. Arndt and J. Amende, Angew. Chem., *43*, 444 (1930); *Organic Syntheses*, Vol. 15, Wiley, New York, 1935, p. 48.

[2] A. R. Jones and W. J. Skraba, Science, *117*, 252 (1953).

[3] *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 461.

[4] *Organic Syntheses*, Vol. 25, Wiley, New York, 1945, p. 28.

[5] *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, pp. 461 and 165.

[6] E. B. Hershberg, E. Schwenk and E. Stahl, Arch. Biochem., *19*, 300 (1948).

[7] J. D. Cox and R. J. Warne, J. Chem. Soc., *1951*, 1896.

[8] R. F. Raffauf, A. L. Farren and G. E. Ullyot, J. Am. Chem. Soc., *75*, 2576 (1953).

PHOSGENE-C^{14}

$$C^*O \xrightarrow[\text{light}]{Cl_2} C^*OCl_2$$

J. L. Huston and T. H. Norris, J. Am. Chem. Soc., *70*, 1968 (1948); M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 155.

A. Procedure

Carbon-C^{14} monoxide is transferred, by Toepler pump on the vacuum line (Figure V,2), into a bulb containing 2.5 times the stoichiometric

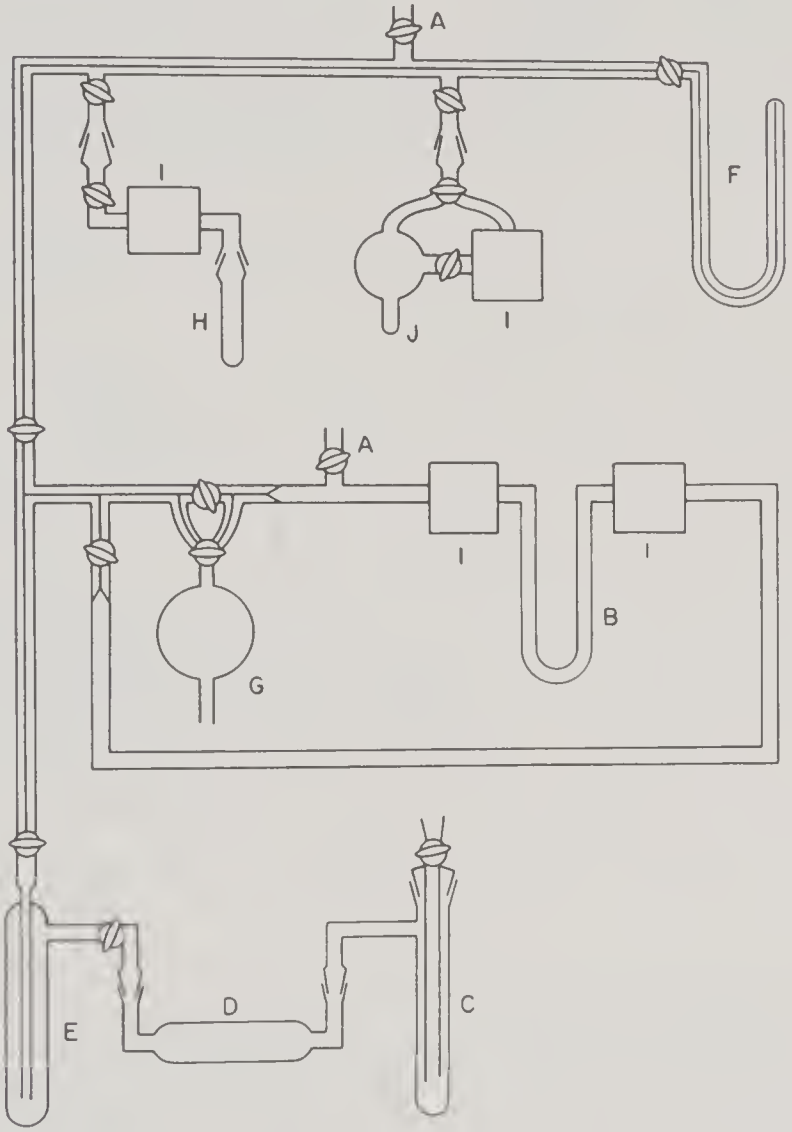


Fig. V,2. Apparatus for preparation of carbon monoxide and phosgene (H. L. Huston and T. H. Norris). A, outlet to vacuum manifold; B, zinc; C, carbon dioxide generator; D, phosphorus pentoxide-on-glass wool; E, cold trap; F, capillary manometer; G, Toepler pump; H, antimony tube; I, spiral cold trap; J, photo-chlorination flask.

amount of chlorine frozen with liquid nitrogen. The bulb is closed off, and the gas mixture is irradiated for about 1 hour with strong visible light (Note 1). The condensable gases are frozen in a liquid nitrogen-cooled trap, and any residual carbon-C^{14} monoxide is pumped off. The condensable gases are distilled into a bulb containing antimony (Note 2); the bulb is removed from the line, thawed and shaken to remove the excess chlorine. The product is distilled into a receiver and again pumped on to remove any residual permanent gases; yield 90–95% or higher (Note 3).

B. Notes

1. The time of irradiation varies with the light source. If a light rich in the ultraviolet (quartz mercury vapor lamp) is used, a reverse reaction is promoted with reduction of yield to 80–85%.

2. Amalgamated copper or mercuric sulfide has been used to remove chlorine. Antimony appears to react slightly with phosgene, producing a trace of carbon monoxide.

3. The preparation of phosgene-C^{14} by a similar procedure has been described by Melville[1] and Stranks.[2] The latter has reported a 6-hour half-time of exchange between carbon-C^{14} monoxide and phosgene at 405° in the dark; the isotope effects and kinetics of photochemical and thermal exchange reactions have been determined.[3]

[1]D. B. Melville, J. G. Pierce and C. W. H. Partridge, J. Biol. Chem., *180*, 299 (1949).

[2]D. R. Stranks, J. Chem. Phys., *21*, 2232 (1953); Trans. Faraday Soc., *51*, 514 (1955).

[3]D. R. Stranks, Trans. Faraday Soc., *51*, 499, 514, 524 (1955).

SILVER CYANAMIDE-C^{14}

$$BaC^*O_3 \xrightarrow[NH_3]{NaN_3} BaNC^*N \xrightarrow{AgNO_3} Ag_2NC^*N$$

S. V. Schuching and F. W. Barnes, Jr., J. Am. Chem. Soc., *72*, 3817 (1950).

A. Procedure

A mixture of 0.200 g. of barium carbonate-C^{14} and 4 g. of sodium azide is heated slowly in a brisk stream of anhydrous ammonia in a six-inch test tube (Note 1). After 60 minutes of heating in such a manner as to maintain a steady, but not too rapid decomposition of the azide, the mixture is cooled in the stream of ammonia. Water is added cautiously to destroy the sodium; then the contents of the tube are dissolved in 150 ml. of water, and the filtered solution is poured slowly into 50 ml. of 0.1 *N* silver nitrate containing 2% ammonia and is set aside for 12 hours. The yellow-green precipitate[1] of silver cyanamide-C^{14} is collected by centrifuging, dissolved in 30 ml. of 3 *N* nitric acid, diluted to 50 ml. with water and filtered. To the filtrate is added 10 ml. of 0.1 *N* silver nitrate, and the mixture is set aside for 12 hours. The mixture slowly is made

alkaline with 5% ammonia; the precipitate is centrifuged and washed free of silver ions with water. The yellow salt is washed with methanol, then with ether, and dried in a desiccator (Note 2). The yield is greater than 94%.

B. Notes

1. The technique is that employed by Adamson, by which sodium cyanide-C^{14} was prepared by heating the same solids in an atmosphere of nitrogen; see potassium cyanide-C^{14}, Method III. The ammonia is dried by passage over potassium hydroxide.

The preparation of silver cyanamide-C^{14} (quantitative) by essentially this procedure is described by Turba; see 2-methylpseudourea-C^{14}.

2. The compound decomposes violently at about 200°. The salt was analyzed for silver and converted to O-methylisourea monohydrochloride, m.p. 126°; yield 90% based on cyanamide. In a control experiment in the absence of barium carbonate, no trace of the silver precipitate appeared.

[1]E. C. Franklin, J. Am. Chem. Soc., *44*, 486 (1922).

CYANAMIDE-C^{14}

METHOD I

$$BaC^*O_3 \xrightarrow{NH_3} BaNC^*N \xrightarrow{H_2SO_4} NH_2C^*N \xrightarrow{NH_4OH} \underset{\underset{NH}{\|}}{NH_2C^*NHC^*N}$$

(a) (b) (c)

S. H. Zbarsky and I. Fischer, Can. J. Research, *27B*, 81 (1949).

A. Procedure

(a) *Barium Cyanamide-C^{14}*. Barium carbonate-C^{14} (3.11 g.), placed in a platinum boat within a quartz combustion tube, is heated by electric furnace to 850° for 3 hours in a stream of pure dry ammonia. The tube is cooled, and the flow of ammonia is discontinued. The boat contains the product in nearly quantitative yield (Note 1).

(b) *Cyanamide-C^{14}*. The boat containing the barium cyanamide-C^{14} is removed to a small beaker and covered with 10 ml. of water. The mixture is cooled in an ice-bath and stirred for 30 minutes to break up any lumps. An amount of cold concentrated sulfuric acid equivalent to the barium is added dropwise, and the mixture is stirred for another hour (Note 2). The mixture is centrifuged, and the supernatant liquid is transferred to a

small round-bottomed flask, the precipitate being thoroughly washed with small volumes of water. The combined solution is evaporated to dryness under reduced pressure at a temperature below 40°. The product is extracted into ether, and the solvent is evaporated. The residue is dried under vacuum over phosphorus pentoxide and again extracted with dry ether. The crystalline residue, left upon evaporation of solvent, is dried as before and then over sodium hydroxide; yield 0.487 g. (73.5%), m.p. 40°.

(c) *Cyanoguanidine-C_2^{14}, (Dicyandiamide-C_2^{14}).* A solution of 0.398 g. of cyanamide-C^{14} in 4 ml. of water containing 2 drops of concentrated ammonium hydroxide (Note 3) is refluxed for 2 hours. The crystals that separate upon cooling are collected and washed with water; a second crop is obtained by evaporating the mother liquor to dryness and recrystallizing the residue from water. The yield is 0.350 g. (88%), and the molar specific activity is unchanged from that of the starting material.

METHOD II

$$\underset{}{NaC^*N} \xrightarrow{Br_2} \underset{(a)}{BrC^*N} \xrightarrow{NH_3} \underset{(b)}{NH_2C^*N}$$

S. Graff, M. Engelman, H. B. Gillespie and A. M. Graff, Cancer Research, *11*, 388 (1951).

A. Procedure

(a) *Cyanogen-C^{14} Bromide* (Note 4). To a stirred mixture of 0.5 ml. of bromine, 0.049 g. (1mmole) of sodium cyanide-C^{14} and 7 ml. of water, in a 25-ml. distilling flask cooled in an ice-bath, is added dropwise a sodium cyanide solution (0.100 g./ml.) until the bromine color disappears (4.33 ml.). The product is distilled, after 15 minutes, into an ice-cooled, three-necked 200-ml. flask containing 10 ml. of ether, complete transfer being assured by distilling an added 10 ml. of ether. The aqueous layer is extracted with four 20-ml. portions of ether, and the combined extracts are dried over sodium sulfate, which is not removed (Note 5). The yield is 84%, determined by treatment of an aliquot with excess standard sodium hydroxide solution and back titration with hydrochloric acid (Note 6).

(b) *Cyanamide-C^{14}* (Note 7). A mixture of the ethereal solution of cyanogen-C^{14} bromide and 5 ml. of 5% methanolic ammonia is stored overnight in a refrigerator, and filtered, and the filtrate is evaporated to dryness under vacuum; yield 0.331 g., 80% based on sodium cyanide (Note 8).

B. Notes

1. In a similar preparation, Bennett[1] has reported that the change in weight of the contents of the boat was almost the theoretical. The exhaust gases contained 1.17% of the initial C^{14} activity as carbon-C^{14} dioxide plus a small amount of alkali-insoluble, radioactive material, which was vented through the hood. A suitable apparatus is a Vycor combustion tube fitted with a thermocouple well extending to a fused silica boat.

2. The pH is tested periodically and maintained at approximately 5.5 by addition of hydrochloric acid.

3. Buchanan[2] has reported that the polymerization takes place quantitatively in alkaline solution up to pH 10, and the velocity of reaction reaches a maximum at pH 9.6. Cyanamide is hydrolyzed quantitatively to urea above pH 12.0.

4. The procedure is a modification of the method of Baum.[3] A tracer study[4] of the synthesis of aliphatic and aromatic nitriles by the reaction of cyanogen bromide with the sodium salts of acetic, propionic and benzoic acids has shown that the carbon dioxide evolved originated in the carboxyl group of the aliphatic acids, but not so in the case of benzoic acid.

5. As cyanogen bromide does not keep well, it is preferable to prepare it just before using. The drying agent is not filtered off because of the appreciable vapor pressure of the product.

6. A similar preparation of cyanogen-C^{13} bromide at the 161-mmole scale has been described by Bendich.[5] The product was dried over phosphorus pentoxide and redistilled; yield 48.4%, b.p. 58–62°.

7. The procedure is essentially that of Bloch.[6]

8. A similar preparation of cyanamide-C^{13}, in 90% yield based on cyanogen-C^{13} bromide, has been described by Bendich.[5]

C. Other Preparations

The preparation of barium cyanamide-C^{14} by Method I has been described by Bennett,[1] Marsh,[7] Holmes[8] and Turba.[9] Barium cyanamide-C^{14} also has been formed in nearly quantitative yield by the fusion of barium carbonate-C^{14} with barium amide (see urea-C^{14}), or sodium azide (see silver cyanamide-C^{14}).

[1]L. L. Bennett, Jr., J. Am. Chem. Soc., 74, 2432 (1952).

[2]G. H. Buchanan and G. Barsky, J. Am. Chem. Soc., 52, 195 (1930).

[3]F. Baum, Ber., *41*, 523 (1908); *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 150.

[4]D. E. Douglas, J. Eccles and A. E. Almond, Can. J. Chem., *31*, 1127 (1953).

[5]A. Bendich, S. S. Furst and G. B. Brown, J. Biol. Chem., *185*, 423 (1950).

[6]K. Bloch, R. Schoenheimer and D. Rittenberg, J. Biol. Chem., *138*, 155 (1941); A. Bendich, J. F. Tinker and G. B. Brown, J. Am. Chem. Soc., *70*, 3109 (1948).

[7]M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 158.

[8]W. L. Holmes, W. H. Prusoff and A. D. Welch, J. Biol. Chem., *209*, 503 (1954).

[9]F. Turba and A. Leismann, Angew. Chem., *65*, 535 (1953).

GUANIDINE-C^{14} HYDROCHLORIDE

$$BaNC^*N \xrightarrow{NH_4NO_3} NH_2C^*(=NH)NH_2 \cdot HNO_3 \xrightarrow{(NO_2)_3C_6H_2ONH_4}$$

(a)

$$(NH_2)_2C^*{=}NH \cdot HOC_6H_2(NO_2)_3 \xrightarrow{HCl} (NH_2)_2C^*{=}NH \cdot HCl$$

(b)

L. L. Bennett, Jr., J. Am. Chem. Soc., *74*, 2432 (1952); Cancer Research, *10*, 644 (1950).

A. Procedure

(a) *Guanidine-C^{14} Nitrate.* The barium cyanamide-C^{14} prepared from 13 mmoles of barium carbonate-C^{14} is ground together with 3.2 g. (40 mmoles) of ammonium nitrate in a 250-ml. centrifuge tube. The mixture is heated at 165° for 20 minutes (Note 1); then the tube is removed from the heating bath and swept with nitrogen, and the mixture again is heated at 165° for 10 minutes while the melt is stirred with a glass rod. The product may be recrystallized from water (Note 2).

(b) *Guanidine-C^{14} Hydrochloride.* The crude guanidine-C^{14} nitrate is stirred with 200 ml. of warm 1.8% aqueous ammonium picrate solution. The picrate, after being allowed to crystallize overnight, is washed by centrifugation with two 20-ml. portions of 0.8% ammonium picrate solution and with two 20-ml. portions of water. The guanidine-C^{14} picrate is dried under vacuum over phosphorus pentoxide (Note 3), then magnetically stirred in ether while dry hydrogen chloride is bubbled in to the point of saturation. The supernatant liquid is removed with a filter stick, and the product is washed with ethereal hydrogen chloride. The product is dissolved in water (Note 4), filtered and evaporated to dryness; yield 0.96 g., 76.3% based on barium carbonate-C^{14} (Note 5).

B. Notes

1. The evolved gases are passed through an alkali trap to remove any carbon-C^{14} dioxide resulting from unreacted barium carbonate-C^{14}.

2. Alcohol is also a satisfactory solvent. A saturated solution of guanidine nitrate in water at 15° contains about 10% of the salt. Guanidine nitrate melts at 213–214°.

3. Guanidine picrate decomposes at 333°.

4. If the product is yellow, the picrate may be removed by passing the aqueous solution through a 1-cm. column of Dowex-1 anion exchange resin, 200–400 mesh.

5. The yield in trial runs was 75–84%. By treating the combined picrate mother liquors with carrier guanidine picrate, 6% of the initial activity can be recovered.

C. Other Preparations

The preparation of guanidine-C^{14} nitrate, guanidine-C^{14} picrate and guanidine-C^{14} hydrochloride, by this method at the 0.7-mmole level, has been described by Marsh, Lane and Salley;[1] yield 61% based on carbon dioxide, purity 93.5%.

Guanidine-C^{14} nitrate (yield 83%, m.p. 206°) has been prepared by Zbarsky and Fischer,[2] by heating cyanoguanidine-C_2^{14}, dicyanodiamide-C_2^{14}, with ammonium nitrate at 175–185°; see Davis.[3]

Guanidine-C^{13} nitrate has been prepared in 66% yield by Bendich,[4] by heating cyanamide-C^{13}, ammonium nitrate and water for 3 hours at 155° in a bomb tube.

[1]M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 158.

[2]S. H. Zbarsky and I. Fischer, Can. J. Research, *27B*, 81 (1949).

[3]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 302.

[4]A. Bendich, S. S. Furst and G. B. Brown, J. Biol. Chem., *185*, 423 (1950); J. S. Blair and J. M. Braham, Ind. Eng. Chem., *16*, 848 (1924).

UREA-C^{14}

METHOD I

$$BaC^*O_3 \xrightarrow{Ba(NH_2)_2} BaNC^*N \xrightarrow[H_2O]{CO_2} NH_2C^*N \xrightarrow{H_2SO_4} (NH_2)C^*O$$

A. Murray III and A. R. Ronzio, J. Am. Chem. Soc., *71*, 2245 (1949); U.S. 2,571,926, Oct. 16, 1951; Chem. Abstracts, *46*, 3561 (1952).

A. Procedure

Dry barium carbonate-C^{14} (0.500 g., 2.53 mmoles) is placed in a 50-ml. long-necked, round-bottomed flask (Note 1). The flask is equipped with

a Dry Ice reflux condenser, connections for vacuum, nitrogen and ammonia lines, and an exhaust through a Drierite guard tube and Bunsen valve. The apparatus is alternately evacuated and filled with nitrogen several times to remove atmospheric carbon dioxide. In a stream of effluent nitrogen, 4–7 g. of shiny barium (Note 2), washed with dry ether, is added along with a crystal of ferric nitrate catalyst, and the apparatus is closed. Liquid ammonia (30 ml.), dried over sodium, is distilled into the reaction flask with Dry Ice cooling, and the mixture is allowed to react until the disappearance of blue color indicates complete conversion of barium to the amide (Note 3). The excess ammonia is distilled off at room temperature through the guard tube and Bunsen valve. The apparatus then is tilted to an angle of 45°, and the mixture is slowly heated with a burner to a dull red, until the fusion ceases to froth and sets to a black mass (Note 4). The mixture is cooled, treated with 30 ml. of water (Note 5) to dissolve the barium cyanamide-C^{14}, and then saturated with carbon dioxide by bubbling the gas through the mixture until the solution no longer tests basic. The barium carbonate is filtered off and washed thoroughly with water. The combined filtrate and washings (80–125 ml.) are treated with 1 ml. of concentrated sulfuric acid, and the solution is evaporated slowly at 40–60° to a syrupy residue (2–3 days, Note 6). The residue is dissolved in 25 ml. of water, then made alkaline with ammonium hydroxide and evaporated to dryness at room temperature under a gentle stream of air (Note 7). The residue is dried under vacuum over calcium chloride, then crushed to a powder and extracted with four 10-ml. portions of warm methanol. The filtered solution is evaporated to dryness in a sublimator, and the residue is vacuum-sublimed[1] at 60–70° at 0.01 mm. (Note 8). The product is recovered from the water-cooled cold finger by solution in methanol and evaporation. The yield is essentially quantitative, m.p. 132–133° (Note 9).

METHOD II

$$KC^*NO \xrightarrow{NH_4OH} NH_4OC^*N \xrightarrow[\Delta]{} (NH_2)_2C^*O$$

E. E. Haley and J. P. Lambooy, J. Am. Chem. Soc., 76, 2926 (1954).

A. Procedure (Note 10)

A mixture of the alkaline solution of potassium cyanate-C^{14} (5.3 ml.) prepared from 0.3284 g. of potassium cyanide-C^{14} by Method I, 0.676 g. of ammonium sulfate and 2.6 ml. of concentrated ammonium hydroxide is heated at 70° for 40 minutes with agitation every 10 minutes (Note 11). The mixture is cooled, the ammonia is distilled under vacuum at room

temperature, and the residual solution is evaporated to dryness (covered petri dish) slowly under vacuum over sulfuric acid. The powdered residue is extracted with four 15-ml. portions of butyl alcohol at 70°, with stirring each time for 30 minutes. The extract is evaporated to dryness under reduced pressure; yield 0.257 g., 80% (Note 12). The radiochemical yield is 86%.

B. Notes

1. The flask is dried overnight at 120°. A diagram of the apparatus is presented by Murray and Ronzio.

2. Later experiments[1] have shown that a weight ratio of barium to barium carbonate-C^{14} of 3.3 to 1 is adequate, with considerable saving of time in forming the amide.

3. If the mixture is left overnight, it is packed in a Dewar flask with Dry Ice to prevent loss of ammonia. A second crystal of catalyst may be added at 12 hours to effect completion of the conversion within 24 hours.

4. The white mixture turns gray, yellow, brown, and finally black before the glass begins to soften.

5. With runs greater than 5 mmoles, it is advisable to add the water to the mixture in an atmosphere of carbon dioxide as a precaution against any possibility of fire.

6. The hydrolysis of cyanamide takes place at the higher acid concentrations that are attained during the later stages of the evaporation.

7. As drying progresses, it is necessary to break up the crust that forms over the remaining liquor.

8. It is advantageous to interrupt the sublimation after several hours, in order to redistribute the residue in the pot by dissolution in methanol and evaporation.

9. Recrystallization of 0.050 g. of urea from 1 ml. of isoamyl alcohol at −30°, without reworking of mother liquors, gives a 91% recovery; the recovery from 7 ml. of acetone at −40° is 85%. Filtration is effected with a filter stick.

A pronounced isotope effect in the oxidation of 9-xanthenylurea-C^{14} and urea-C^{14} nitrate has been reported by Armstrong.[2]

10. The procedure is a modification of the method of Wöhler.[3]

11. The apparatus is a 13-ml., pressure-sealed, screw-cap tube, fitted with a sealed-in thermometer and heated with a water-bath.

12. The purity is 94%, determined by the urease method. Four trial runs have given yields of 79, 83, 79 and 84% with purities of 97, 98, 95 and 97%, respectively. The relative rates of hydrolysis of urea containing C^{12}, C^{13} and C^{14} in the presence of urease has been determined by Schmitt.[4]

C. Other Preparations

By Method II is prepared[5] urea-C^{14}-N^{15} from ammonium-N^{15} cyanate-C^{14}; yield 42%, m.p. 132–133°. Biuret-C_2^{14}-N_3^{15} is formed as a by-product in 10–15% yield.

Urea-C^{14} has been prepared by Zbarsky[6] by hydrolysis of cyanamide-C^{14} (0.3 g.) in 91% yield (67.4% from barium carbonate without isolating the intermediate).

Urea-C^{14} has been prepared in 40% yield at the millimole scale by Myerson.[7,8] Carbon-C^{14} dioxide and ammonia were combined, quantitatively forming ammonium carbamate-C^{14}, which then was heated at 135° in a sealed tube to form the equilibrium mixture with 40% conversion to urea-C^{14}, m.p. 131.5° without recrystallization. The yield by this method has been raised to about 80% on the 2-g. scale by employing high pressure and temperature, as reported by Skipper,[8] 2800 p.s.i., 200°, 3–4 hours.

Urea-C^{11} has been prepared in quantitative yield by Yankwich,[9] by bubbling ammonia through a solution of phosgene-C^{11} in toluene. The preparation of many substituted ureas is possible by this method, as the reaction of phosgene with primary and secondary amines is general.

[1]C. W. Bills and D. L. Williams, private communication.

[2]W. D. Armstrong, L. Singer, S. H. Zbarsky and B. Dunshee, Science, *112*, 531 (1950).

[3]F. Wöhler, Pogg. Ann., *12*, 253 (1828).

[4]J. A. Schmitt, A. J. Myerson and F. Daniels, J. Phys. Chem., *56*, 917 (1952); J. A. Schmitt and F. Daniels, J. Am. Chem. Soc., *75*, 3564 (1953).

[5]D. L. Williams and A. R. Ronzio, J. Am. Chem. Soc., *74*, 2407 (1952).

[6]S. H. Zbarsky and I. Fischer, Can. J. Research, *27B*, 81 (1949).

[7]A. L. Myerson, J. Am. Chem. Soc., *74*, 2436 (1952); A. L. Myerson and F. Daniels, Science, *108*, 676 (1948).

[8]H. E. Skipper, C. E. Bryan and O. S. Hutchison, U. S. Atomic Energy Commission, Isotopes Division Circular C-8, September, 1947; P. H. Groggins, *Unit Processes in Organic Synthesis*, 2nd Ed., McGraw New York, 1938, p. 357.

[9]M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 157.

THIOUREA-C^{14}

$$BaNC^*N \xrightarrow[Ba(OH)_2]{H_2S} (NH_2)_2C^*S$$

C. W. Bills and A. R. Ronzio, J. Am. Chem. Soc., *72*, 5510 (1950).

A. Procedure

In a mixture of 25 ml. of water and 0.3 g. of barium hydroxide is dissolved the crude barium cyanamide-C^{14} prepared[1] from 1.52 mmoles of barium carbonate-C^{14} (see urea-C^{14}). Hydrogen sulfide is bubbled slowly through the solution for 2 hours at room temperature. The flask is tightly stoppered (Note 1) and heated for 20 hours in a water-bath at 85°. The barium is removed as the carbonate by saturating the mixture with carbon dioxide (Note 2), filtering and washing with alcohol. The filtrate is evaporated to dryness (Note 3), and the residue is extracted several times into alcohol and treated with charcoal. The filtrate is evaporated to dryness in a vacuum sublimator (0.2228 g.), and the product is sublimed at 70–90° at 0.03 mm (Note 4); yield 97%, m.p. 171–173° (Note 5).

B. Notes

1. The stopper is wired tightly to the flask.

2. The mixture gives a neutral reaction to indicator paper in about 20 minutes.

3. The use of an infrared lamp and a gentle air stream facilities the evaporation.

4. The yield drops to 87%, m.p. 171–172°, if purification is effected by two recrystallizations from 2–3 ml. of 1-butanol at 0°. Any sublimed sulfur is left on the condenser when the thiourea is dissolved off with methanol.

5. The purity of thiourea prepared in trial runs was found by Williams[2] to be 99.4–99.8%, by iodimetric titration according to the method of Werner.[3]

C. Other Preparations

The preparation by the described procedure has been reported by Holmes[4] and Bennett (90–122% apparent yield).[8]

Thiourea-C^{14} has been prepared in a similar manner by Graff,[5] by saturating an ammonium hydroxide solution of cyanamide-C^{14} with hydrogen sulfide; yield 77.5% based on sodium cyanide.

Thiourea-C^{14} has been prepared in 61.4% yield by Zbarsky,[6] by treating an aqueous mixture of cyanamide-C^{14}, antimony pentasulfide and hydrochloric acid with hydrogen sulfide for 2 hours on a steam-bath according to the method of Plentl.[7]

[1] A. Murray III, and A. R. Ronzio, J. Am. Chem. Soc., *71*, 2245 (1949).

[2] D. L. Williams and A. R. Ronzio, Atomic Energy Commission Report, AECU-1394; Nuc. Sci. Abstracts, *5*, 5145 (1951).

[3] E. A. Werner, J. Chem. Soc., *101*, 2168 (1912).

[4]W. L. Holmes, W. H. Prusoff and A. D. Welch, J. Biol. Chem., *209*, 503 (1954).

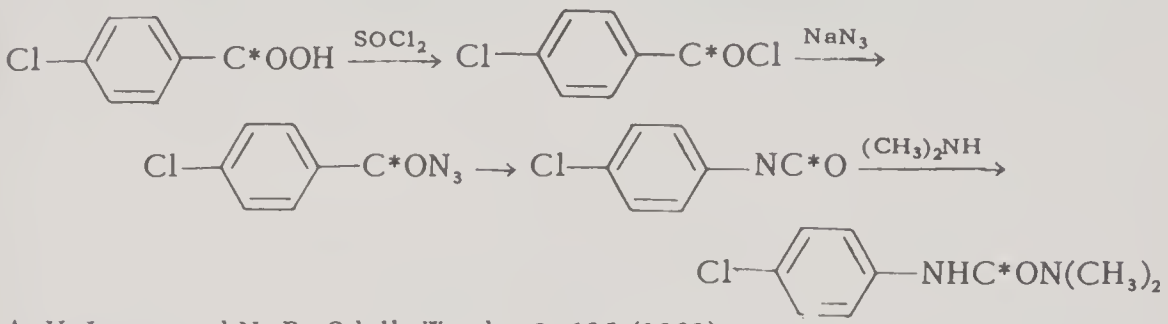
[5]S. Graff, M. Engelman, H. B. Gillespie and A. M. Graff, Cancer Research, *11*, 388 (1951).

[6]S. H. Zbarsky and I. Fischer, Can. J. Research, *27B*, 81 (1949).

[7]A. A. Plentl and R. Schoenheimer, J. Biol. Chem., *153*, 203 (1944).

[8]L. L. Bennett, Jr. and H. T. Baker, Southern Research Institute, Birmingham, Ala., private communication.

3-(*p*-CHLOROPHENYL)-1,1-DIMETHYLUREA-2-C^{14}



A. V. Logan and N. R. Odell, Weeds, *2*, 135 (1953).

A. Procedure

A mixture of 0.1222 g. (0.78 mmole) of *p*-chlorobenzoic-C^{14} acid, 2.0 ml. of dry toluene and 7 ml. of purified thionyl chloride is refluxed for 1 hour. The excess thionyl chloride is removed by distilling 7 ml. of solvent and two 5-ml. portions of added toluene. To the toluene solution (2 ml.) of *p*-chlorobenzoyl-C^{14} chloride is added 0.1017 g. (1.564 mmoles) of activated sodium azide, and the mixture is refluxed for 24 hours (Note 1). To the resulting solution of *p*-chlorophenyl isocyanate-C^{14} is added an excess of dimethylamine with ice-bath cooling (Note 2). The solution is warmed for 10 minutes to remove the excess amine, then filtered, and the residue is washed with 20 ml. of acetone. The combined filtrate is evaporated, and the residue is dissolved in dilute hydrochloric acid (1:4). The solution is treated with dilute sodium hydroxide to precipitate the product; yield 56.6% based on barium carbonate, m.p. 171° (Note 3). The radiochemical yield is 53.6% based on barium carbonate-C^{14}.

B. Notes

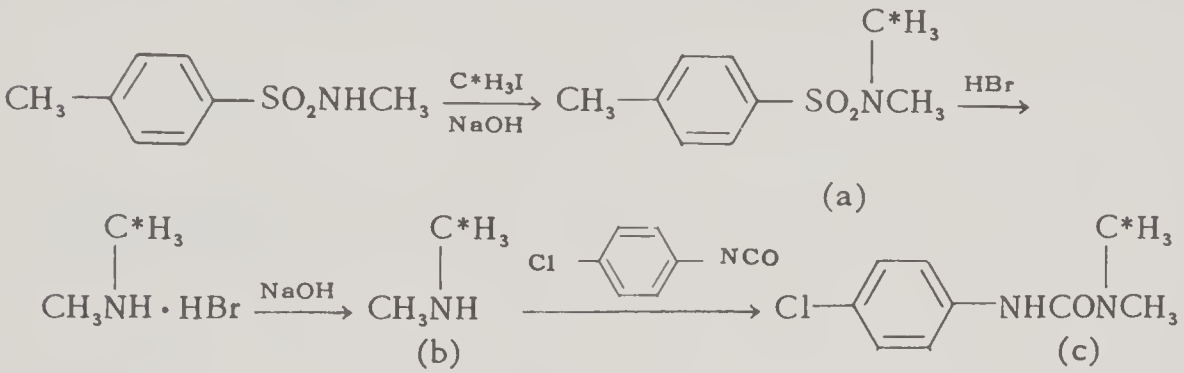
1. The isocyanate is prepared by a modification of the method of Barrett and Porter;[1] also see the Curtius rearrangement.[2] The preparation of activated sodium azide is described by Smith.[2]

2. The procedure is that of Bucha and Porter.[3]

3. An authentic sample recrystallized from methanol melts at 170.5–171.5°. The compound is essentially neutral, stable, insoluble in water and only moderately soluble in most organic solvents.

[1]E. W. Barrett and C. W. Porter, J. Am. Chem. Soc., 63, 3434 (1941).
[2]*Organic Reactions*, Vol. III, Wiley, New York, 1946, Chapter 9, p. 382.
[3]H. C. Bucha and C. W. Porter, Science, *114*, 493 (1951); C. Kao, H. Fang and P. P. T. Sah, J. Chinese Chem. Soc., *3*, 137 (1935); Chem. Abstracts, *29*, 6172 (1935).

3-(*p*-CHLOROPHENYL)-1,1-DIMETHYL-C_1^{14}-UREA



N. E. Searle and H. E. Cupery, J. Org. Chem., *19*, 1622 (1954).

A. Procedure

(a) N,N-*Dimethyl*-C_1^{14}-p-*toluenesulfonamide*. To a stirred solution of 0.814 g. (4.4 mmoles) of *N*-methyl-*p*-toluenesulfonamide (Note 1), 0.176 g. (4.4 mmoles) of sodium hydroxide, 5.2 ml. of water and 4 ml. of methanol, in a 50-ml. flask at 25°, is added 0.543 g. (3.83 mmoles) of methyl-C^{14} iodide by transfer in a slow stream of nitrogen. The effluent gas is passed through a U-tube cooled with liquid nitrogen to trap any unreacted methyl-C^{14} iodide, which is then passed back into the reaction mixture. Complete reaction of the iodide is achieved by repeating the procedure five times, while the reaction temperature is progressively raised to 55°. The mixture is treated with 1 ml. of 10% sodium hydroxide solution, and the methanol is removed by distillation (Note 2). The residue is cooled to crystallize the product, the aqueous solution of excess starting amide is removed through a filter stick, and the product is washed with two portions of 2% sodium hydroxide solution and two portions of water.

(b) *Dimethylamine*-C_1^{14}. A mixture of the crude amide and 8 ml. of 48% hydrobromic acid is refluxed for 1.5 hours under nitrogen. To the resultant ice-cooled, stirred solution of dimethylamine-C_1^{14} hydrobromide is added slowly 20 g. of 50% aqueous sodium hydroxide. The liberated amine is swept by a slow stream of nitrogen through the reflux condenser and a tower packed with potassium hydroxide pellets into a 50-ml. flask containing

6 ml. of dry hexane, which is cooled by Dry Ice-acetone and guarded by a U-trap cooled with liquid nitrogen and protected from atmospheric moisture (Note 3). The reaction mixture is heated slowly to reflux during 1.5 hours, and refluxing is continued for 15 minutes to insure complete transfer of the product.

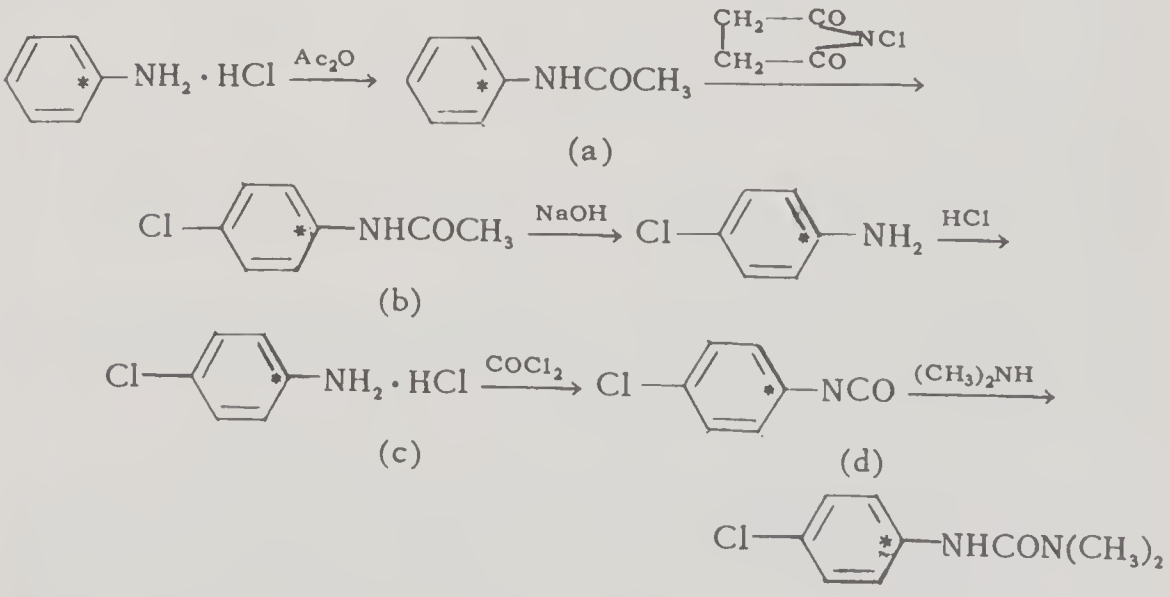
(c) *3-(p-Chlorophenyl)-1,1-dimethyl-C_1^{14}-urea.* To the solution of dimethylamine-C_1^{14} in hexane is added a solution of 0.82 g. (5.35 mmoles) of freshly distilled *p*-chlorophenyl isocyanate in 9 ml. of dry hexane, followed by the condensate in the liquid nitrogen trap (Note 4). The mixture is warmed gradually to 45° and cooled, and the liquor is drawn off through a filter stick under anhydrous conditions (see Note 3). The product is washed with two portions of hexane and dried over phosphorus pentoxide; yield 0.275 g. (36.3%, Note 5), m.p. 170–171°.

B. Notes

1. Directions are given by Lukes[1] for preparing the amide.
2. The distillate is essentially nonradioactive.
3. Atmospheric moisture contaminates the product of the next step with 4,4′-dichlorocarbanilide.
4. A stream of nitrogen is used to effect the transfer of amine condensate.
5. The yield is usually 80–85% in the absence of accidental loss.

[1]R. Lukes and J. Preucil, Collection Czeckoslov. Chem. Commun., *10*, 384 (1938); Chem. Abstracts, *33*, 983 (1939).

3-(*p*-CHLOROPHENYL-1-C^{14})-1,1-DIMETHYLUREA



N. E. Searle and H. E. Cupery, J. Org. Chem., *19*, 1622 (1954).

A. Procedure

(a) *Acetanilide-1′-C^{14}.* A stirred mixture of 1.36 g. (10.5 mmoles) of aniline-1-C^{14} hydrochloride, 16 ml. of carbon tetrachloride (Note 1) and 3.0 g. of acetic anhydride is refluxed for 25 minutes, then treated with 3.0 g. of acetic anhydride and refluxed for 20 minutes (Note 2). The cooled mixture is treated with 10 ml. of methanol, and the methyl acetate formed is slowly distilled off, the by-product water being removed by azeotropic distillation with 30 ml. of carbon tetrachloride added in several portions (Note 3).

(b) *4′-Chloroacetanilide-1′-C^{14}.* To the dry crude acetanilide-1′-C^{14} is added 1.55 g. (11.6 mmoles) of *N*-chlorosuccinimide (Note 4) and 7 ml. of acetic acid (Note 5). The mixture is warmed gently to initiate an exothermic reaction, which raises the temperature to 60° while the flask is cooled with an ice-bath. The stirred mixture is then maintained at 82° until only a trace of chlorinating reagent remains (Note 6). The acetic acid is removed by evaporation under vacuum, and the residual paste is washed with several 10-ml. portions of hexane (Note 7) and with 5 ml. of water (Note 8).

(c) p-*Chloroaniline-1-C^{14} Hydrochloride.* To the aqueous paste of the crude 4′-chloroacetanilide-1′-C^{14} is added 2.1 g. (5.25 mmoles) of sodium hydroxide in 4 ml. of water and 10 ml. of methanol. A few drops of 5% aqueous sodium bisulfite are added to decompose any traces of *N*-chlorosuccinimide (Note 9), and the mixture is refluxed for 1.5 hours, stored for 1.5 hours, then diluted with 10 ml. of water and heated to distill off the methanol. The *p*-chloroaniline-1-C^{14} is isolated by extraction with methylene chloride, and the concentrated extract is diluted with 25 ml. of dioxane and distilled until the vapor temperature reaches 100° (Note 10). The cooled solution is saturated at 5° with anhydrous hydrogen chloride to precipitate the product as fluffy crystals (Note 11).

(d) p-*Chlorophenyl-1-C^{14} Isocyanate.* Through the stirred slurry of hydrochloride crystals at 75° is passed a stream of dry, chlorine-free phosgene (Note 12) for 20 minutes. Hydrogen chloride and excess phosgene are removed from the clear, pale amber solution of product by slow distillation of a portion of the solvent.

(e) *3-(p-Chlorophenyl-1-C^{14})-1,1-dimethylurea.* An excess of anhydrous dimethylamine is passed into the cooled solution of the isocyanate (10°); then the mixture is heated for a short time at 55° and evaporated under vacuum. The residue is dissolved in 75 ml. of methylene chloride, and the solution is decolorized with charcoal, concentrated to 5 ml. in a stream of nitrogen, diluted with 20 ml. of hexane and chilled. The product is collected, washed with 10 ml. of cold hexane and dried under a heat lamp while air is slowly drawn through the sintered-glass funnel; yield 1.164 g. (56%), m.p. 170.2–171.3° (sintered 169.5°) (Note 13).

B. Notes

1. The carbon tetrachloride prevents formation of the oily diacetanilide.

2. Hydrogen chloride is evolved.

3. In trial runs the yield of essentially pure product was quantitative.

4. The *N*-chlorosuccinimide is recrystallized from acetic acid, m.p. 147.5–148.5°. A 10% excess of the reagent gives the best yield of the desired *p*-isomer.

5. Acetic acid combines the most desirable properties of a number of solvents studied.

6. At 45 minutes only a faintly positive test is obtained with moist starch-potassium iodide paper.

7. Evaporation of the filtered extract gives crude 2′-chloroacetanilide-1′-C^{14}; yield 0.35 g. (20%).

8. The by-product succinimide is very water-soluble.

9. The presence of unreacted *N*-chlorosuccinimide during the hydrolysis step results in highly colored aniline oxidation products. The reagent may be removed, alternatively, by warming the mixture slightly with isopropyl alcohol, the alcohol being oxidized to acetone.

10. Distillation removes traces of water.

11. 4,4′-Dichlorocarbanilide may be formed in the next step if the *p*-chloroaniline is not completely converted to the hydrochloride.

12. Phosgene is purified by bubbling through cotton seed oil and sulfuric acid.[1]

13. Evaporation of the wash liquors gives crude 3-(*o*-chlorophenyl-1-C^{14})-1,1-dimethylurea; yield 0.242 g. (12%), oil crystallizing upon standing.

[1]R. L. Shriner, W. H. Horne and R. F. B. Cox, *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 453.

ETHYL CARBAMATE-C^{14}
(C_1^{14}-Urethan)

$$(NH_2)_2C^*O \xrightarrow{HNO_3} (NH_2)_2C^*O \cdot HNO_3 \xrightarrow[NaONO]{C_2H_5OH} NH_2C^*OOC_2H_5$$
(a) (b)

H. E. Skipper, C. E. Bryan and O. S. Hutchison, U. S. Atomic Energy Commission, Isotopes Division Circular C-8, Sept., 1947.

A. Procedure

(a) *Urea-C^{14} Nitrate.* To a concentrated aqueous solution of 2.0 g. of urea-C^{14} is added dropwise an excess of concentrated nitric acid. The precipitated product is collected and dried in a vacuum desiccator over potassium hydroxide; yield 100%.

(b) *Ethyl Carbamate-C*^{14}. Urea-C^{14} nitrate is converted to ethyl carbamate-C^{14} by an adaptation of the method of Guerci,[1] which follows. The yield is 40%, m.p. 49–50°. The benzene-insoluble material is oxidized to carbon-C^{14} dioxide for recovery of isotope.

The apparatus consists of a flask connected with a reflux condenser, a calcium chloride tube and a wash bottle containing sufficient mercury to maintain a pressure of 0.5 atmospheres. To a mixture of dry urea nitrate (1 mole) and absolute alcohol (8 moles) at 60–70° is added dry sodium nitrite (1 mole) in small portions. After refluxing for an additional hour, the solvent is distilled rapidly at reduced pressure. The cooled, solidified residue is extracted with three portions of hot benzene, and the extract is concentrated and cooled, yielding colorless, shining tablets, m.p. 49–50° (80% yield).

[1]L. Guerci, Giorn. chim. ind. applicata, *4*, 60 (1921); through Chem. Abstracts, *16*, 2481 (1922); A. Andreocci, Ber., *25*, 639C (1892).

2-CHLORO-1-(CHLOROMETHYL)ETHYL *N,N*-DIETHYLCARBAMATE-C^{14}

$$HOCH(CH_2Cl)_2 \xrightarrow[C_6H_5N(CH_3)_2]{C^*OCl_2} ClC^*OOCH(CH_2Cl)_2 \xrightarrow{(C_2H_5)_2NH}$$

(a)

$$(C_2H_5)_2NC^*OOCH(CH_2Cl)_2$$

(b)

A. Murray III, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, unpublished work.

A. Procedure (Note 1)

(a) *2-Chloro-1-(chloromethyl)ethyl Chloroformate-C*^{14}. To a stirred, Dry Ice-cooled (Note 2) solution of 7.0 ml. of dry toluene and 0.2911 g. (2.94 mmoles) of phosgene-C^{14} is added dropwise during 15 minutes a solution of 0.294 ml. (3.09 mmoles, 1.3506 g./ml.) of 1,3-dichloro-2-propanol and 0.40 ml. (3.15 mmoles) of dry *N,N*-dimethylaniline in 3.0 ml. of dry toluene (Note 3). The solution is stirred overnight at room temperature (Note 4).

(b) *2-Chloro-1-(chloromethyl)ethyl* N,N-*Diethylcarbamate-C*^{14}. To the stirred (Note 5), Dry Ice-cooled mixture of the chloroformate under dry nitrogen is added during 15 minuets a solution of 0.91 ml. (8.82 mmoles) of dry diethylamine in 5 ml. of absolute ether (Note 6). The mixture is stirred for 4 hours and warmed slowly to room temperature, then stored

for 24 hours, filtered and washed thoroughly with absolute ether.. The combined filtrate is evaporated at room temperature under reduced pressure, and the oily residue is stirred with 0.417 g. (3.31 mmoles) of oxalic acid (dihydrate) in 4.0 ml. of absolute ethanol. The slurry is evaporated to dryness under reduced pressure, and the residue is stirred with 50 ml. of petroleum ether (Note 7); anhydrous calcium chloride (40 mesh) is added, and the mixture is stirred for 1.5 hours, then filtered with the aid of five 5-ml. portions of wash solvent. The combined colorless filtrate is concentrated, and the crude product is extracted into hexane solution (Note 8), which is percolated through a thin bed of Norit and evaporated under vacuum; yield 0.6147 g. (89.7% based on barium carbonate), radiochemical yield 80.3% (Note 9). The product is stored best in hexane solution in a sealed ampoule (Note 10).

B. Notes

1. The procedure is a modification of the method of Raiford.[1] Since it is undesirable to purify a labeled liquid product by fractional distillation at the semimicro scale, excess reagents, in this case, are separated through solubility considerations.

2. Low temperature favors formation of the chloroformate rather than the carbonate.[2]

3. 1,3-Dichloro-2-propanol is dried over Drierite and fractionated through a 6-bulb Snyder column, b.p. 70.5–71.8° (14.5 mm.); *N,N*-dimethylaniline is similarly dried and distilled in the dark, b.p. 183.3–183.8° (585 mm.).

4. The bright green solution turns pale yellow in 45 minutes. The chloroformate ester boils at 183–185° (585 mm.); the derivative[3] 2-chloro-1-(chloromethyl)ethyl carbamate melts at 80–82°.

5. A permanent magnet is used to clamp the magnetic stirring bar to the upper wall of the flask, so that a mercury-sealed stirrer may be used to handle the heavy salt precipitate.

6. Diethylamine is dried over Drierite and fractionated, b.p. 47.0–47.7°.

7. Although the carbamate is miscible with nearly all common solvents, a slight turbidity forms in hexane (and petroleum ether) solution and deposits upon the glass as a fluorescent oily film during brief standing. This unknown contaminant, insoluble in fresh solvent, was present in an authentic specimen. Oxalic acid is insoluble in the hydrocarbon solvent.

8. A small amount of nonisotopic amine salt crystallizes out.

9. No solvent system was found capable of giving more than one radioactive band by one-dimensional paper chromatography. The extreme solubility of the carbamate in nearly all of the common solvents, polar as well as nonpolar, causes the band to move with the solvent fronts.

The infrared spectrum of the product obtained in trial preparations agreed closely with that of an authentic specimen; n_D^{20} 1.4645, b.p. 123-124° (9 mm.). Samples were recovered unchanged by column chromatography in: benzene on neutral alumina, Brockmann IV (I probably causes some decomposition), 92% recovery; hexane on activated silica, 98% recovery.

10. Advantage is taken of the dispersion in inert solvent to minimize decomposition by radiolysis.

[1]L. C. Raiford and G. O. Inman, J. Am. Chem. Soc., *56*, 1586 (1934); R. E. Oesper, W. Broker and W. A. Cook, J. Am. Chem. Soc., *47*, 2609 (1925); J. S. Pierce and R. Adams, J. Am. Chem. Soc., *45*, 790 (1923); C. S. Marvel and W. A. Noyes, J. Am. Chem. Soc., *42*, 2276 (1920).

[2]C. E. Slimowicz and E. F. Degering, J. Am. Chem. Soc., *71*, 1043 (1949).

[3]*Organic Syntheses*, Vol. 23, Wiley, New York, 1943, p. 13.

1-METHYLETHYL-1-C^{14} CARBANILATE
(1-Methylethyl-1-C^{14} *N*-Phenylcarbamate)

$$(CH_3)_2C^*O \xrightarrow{LiAlH_4} (CH_3)_2C^*HOH \xrightarrow{C_6H_5NCO} C_6H_5NHCOOC^*H(CH_3)_2$$
(a) (b)

A. V. Logan and J. Murray, J. Am. Chem. Soc., *74*, 2436 (1952).

A. Procedure

(a) *2-Propanol-2-C^{14}*. To the 2-propanone-2-C^{14} prepared from 1.99 mmoles of barium acetate-1-C^{14}, frozen with liquid nitrogen in a flask fitted with an addition tube and a low-temperature (Dry Ice-isopropyl alcohol) reflux condenser, is added slowly 2 ml. of absolute ether which is frozen over the acetone. A solution of 0.1 g. of lithium aluminum hydride[1] in 2 ml. of absolute ether, under dry nitrogen, is added in a similar manner with freezing. The freezing bath is replaced by a Dry Ice-isopropyl alcohol bath so that slow diffusion of the melting layers effects smooth reduction of the ketone. The reaction intermediate is freed from solvent by evaporation under vacuum, then decomposed by the careful addition of 4 ml. of dilute hydrochloric acid. The evolved hydrogen is vented through a liquid nitrogen-cooled trap containing 4 g. of recalcined and outgassed calcium oxide. The alcohol-water azeotrope is vacuum-distilled fractionally into the trap, where the product is dried for 30 minutes over the oxide at room temperature, and then collected by distillation.

(b) *1-Methylethyl-1-C^{14} Carbanilate*. A mixture of the 2-propanol-2-C^{14} and 0.3 ml. of phenyl isocyanate is heated under reflux for 3 hours at

80°. The mixture is taken up in 10 ml. of boiling heptane, filtered, and cooled to crystallize the product; yield 0.160 g., m.p. 84–85°. A second crop of 0.164 g., m.p. 79–80°, is recovered from the mother liquor.

B. Other Preparations

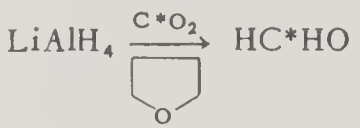
The preparation of 2-propanol-2-C^{14} by hydrogenation of 2-propanone-2-C^{14} in purified dioxane over Raney nickel (5 hours, 40 p.s.i.), has been reported by Coon.[2] The carbinol was distilled and converted to 2-bromopropane-2-C^{14} by treatment with phosphorus tribromide; yield 50% based on acetic acid.

[1]R. F. Nystrom and W. G. Brown, J. Am. Chem. Soc., 69, 1197 (1947).
[2]M. J. Coon and S. Gurin, J. Biol. Chem., *180*, 1159 (1949).

CARBONYL COMPOUNDS

FORMALDEHYDE-C^{14}

METHOD I



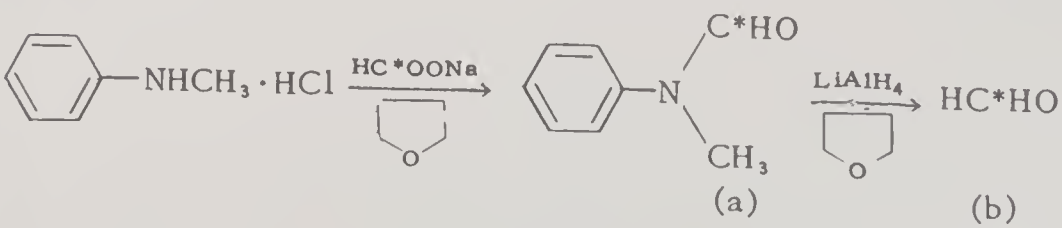
F. Weygand and H. Linden, Angew. Chem., *66*, 174 (1954); Z. Naturforsch., *9b*, 682 (1954).

A. Procedure

To a solution of 1 mmole of lithium aluminum hydride in 4–5 ml. of anhydrous tetrahydrofuran, frozen with liquid nitrogen, is added 1 mmole of carbon-C^{14} dioxide by vacuum transfer. The reaction flask is sealed with a stopcock and shaken at 0° for 30 minutes. The mixture is hydrolyzed by the addition of methanol and then dilute acid. The yield, determined by formation of the dimedon derivative, is 65% (Note 1).

In cases where the tetrahydrofuran interferes with use of the product, the solvent is removed at 0° under high vacuum so that the total reaction time is still about 30 minutes. The flask is filled with nitrogen, and the dry complex is hydrolyzed at −80° by the addition of 0.5 ml. of methanol.

METHOD II



(a) (b)

F. Weygand and H. Linden, Angew. Chem., 66, 174 (1954).

A. Procedure (Note 2)

(a) *N-Methylformanilide-1-C*^{14} (Note 3). The anilide is prepared in 90–95% yield at the 3-mmole level by heating sodium formate-C^{14} with *N*-methylaniline hydrochloride at 150° and extracting the crude product with tetrahydrofuran.

(b) *Formaldehyde-C*^{14}. To the stirred solution (about 10% of crude *N*-methylformanilide-1-C^{14} in tetrahydrofuran, with ice-salt bath cooling, is added a solution of 0.5 molecular amounts of lithium aluminum hydride in tetrahydrofuran (Note 4). The cold mixture is stirred for 1 hour, then hydrolyzed with dilute acid, and the product is isolated by extraction or steam distillation; yield 75–80%, or 64–72% based on barium carbonate (Note 5).

METHOD III

$$C^*H_3OH \xrightarrow[Ag]{O_2} HC^*HO$$

F. Weygand and G. Schaefer, Ber., *85*, 307 (1952); Naturwiss., *38*, 432 (1951).

A. Procedure

The apparatus consists of a source of dry air which feeds under constant pressure into a steam-heated chamber fitted with two pressure-equalizing burettes, arranged in parallel for dispensing methanol solutions, and attached to a vertical quartz tube containing a heating element and finely powdered electrolytic silver catalyst. The bottom of the tube is attached through a spiral air-condenser to a receiver and a gas washing tower fitted with a dropping funnel and drain; see Figure VI, 1. The optimum operating conditions for each individual apparatus are arrived at only by experiment (Note 6).

With an air flow of 39 l. per hour (Note 7), a solution of 19 g. of methanol and 19 g. of water is passed through the heated catalytic dehydration apparatus over a period of 1 hour (Note 8). The receiver is changed, and a solution of 6 g. of methanol-C^{14} and 6 g. of water is passed from the second burette through the now activated catalyst (Note 9). The yield of formaldehyde-C^{14} is 4.75 g., 85.8% as a 26% aqueous solution (Note 10).

METHOD IV

$$C^*H_3OH \xrightarrow[Cat.]{O_2} HC^*HO$$

A. Murray III, C. W. Bills and A. R. Ronzio, J. Am. Chem. Soc., *74*, 2405 (1952); H. R. V. Arnstein, Nature, *164*, 362 (1949); Biochem. J., *49*, 439 (1951).

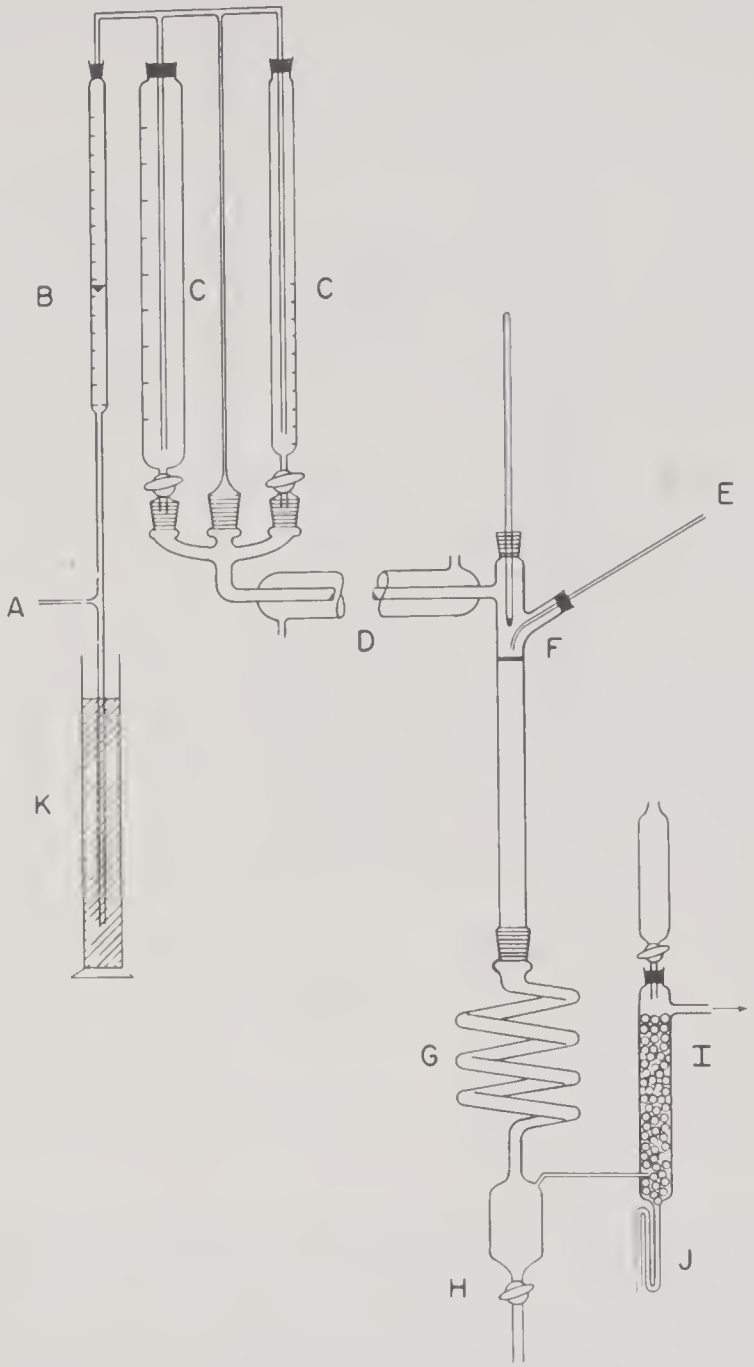


Fig. VI, 1. Apparatus for preparation of formaldehyde (F. Weygand and G. Schaefer). A, inlet from source of dry air pressure; B, rotameter; C, pressure-equalizing burettes; D, steam heated chamber; E, thermo-element; F, catalyst; G, air condenser; H, receiver; I, gas-washing tower; J, drain; K, pressure regulator.

A. Procedure (Note 11)

The apparatus (Figure VI, 2) consists of a source of purified dry air which feeds under constant pressure (Note 12) into a vertical tube containing methanol-C^{14} and maintained at constant temperature by a water-bath at 20°. Separated from this tube by a coarse sintered glass disc is a 4.0-cm. section of 12 mm. Pyrex tubing filled with 3-mm. glass beads,

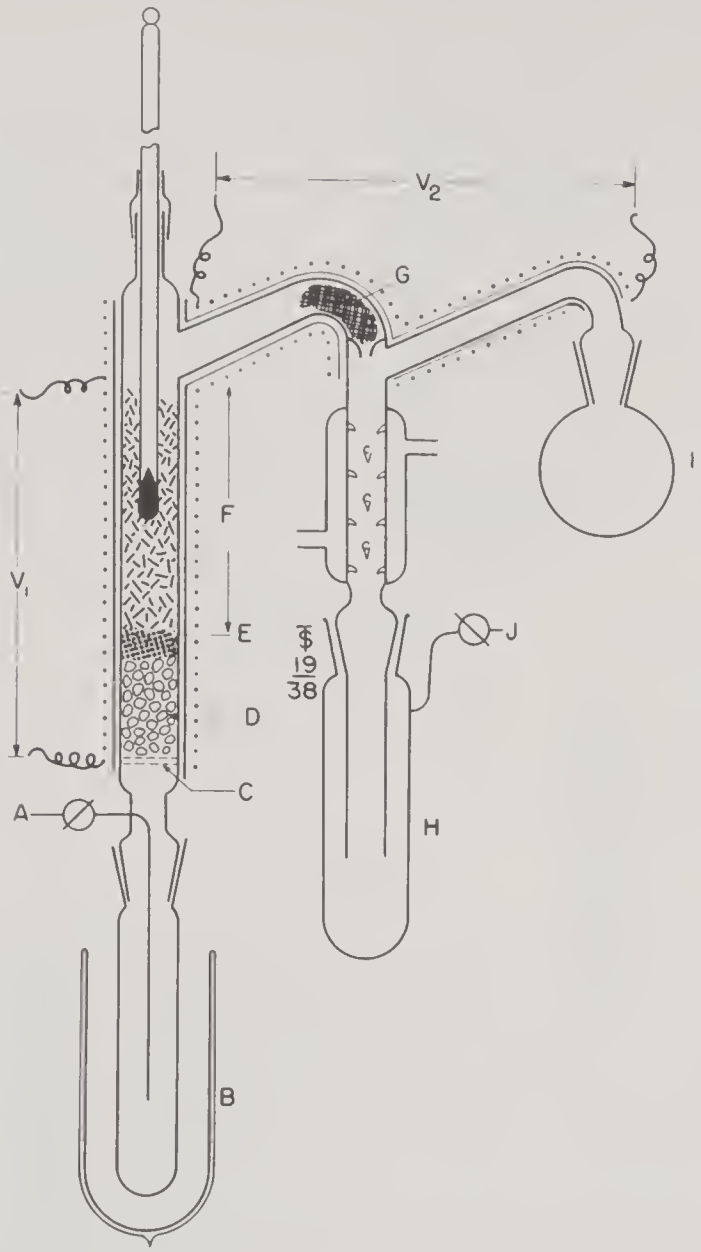


Fig. VI, 2. Apparatus for the preparation of formaldehyde (A. Murray III, C. W. Bills and A. R. Ronzio). A, inlet from source of constant dry air; B, methanol vaporizer; C, sintered disc; D, beads; E, asbestos mat; F, catalyst; G, glass wool; H, receiver; I, steam generator; J, air outlet to drying train and Mariotte flask; V, Variac connections.

assuring thorough mixing of air and methanol vapor. The oxidation chamber consists of an electrically heated (Note 13) 10-cm. section of the tubing, containing 5 g. of catalyst held in place above the beads by a thin mat of asbestos. The bulb of a 500°-range thermometer extends into the middle of the catalyst, which is held in place by a top plug of glass wool. The side arm from the reactor is joined by one from a steam generator (Note 14) at the top of a short vertical water-cooled condenser. The receiver inlet tube is the largest that passes through a 19/38 joint and is of such length as to clear the surface of 25 ml. of distillate in the receiver, which is cooled with Dry Ice chips. The air outlet is con-

nected, through a calcium chloride tube and safety trap, to a Mariotte flask so that the rate of air flow may be determined at any time by measuring the rate of water displacement (Note 15).

The molybdenum oxide-ferric oxide catalyst is prepared by covering 30 g. of acid-washed, porous tile (−20 + 40 mesh) with an aqueous solution of 4.38 g. of $Fe(NO_3)_3 \cdot 9H_2O$ and boiling briefly in a casserole. A concentrated solution of 12.28 g. of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 15 ml. of warm ammonium sulfide solution is added, and the mixture is boiled to dryness and ignited with a Meeker burner. To ensure complete oxidation, the catalyst is heated for 20 minutes in a stream of oxygen in a quartz tube at 600–700° (Note 16).

The optimum operating conditions for any new apparatus and catalyst are determined by studying the effects of temperature and rate of air flow, the catalyst temperature being much the more critical of the two. In a typical run the catalyst is maintained at 375 ± 2°, and the air flow is adjusted to 200 ± 25 ml./min. (Note 17). After steam has been generated (gently) for 5 minutes, boiling is discontinued momentarily while the empty alcohol tube is replaced by a duplicate containing 0.0392–0.0985 g. (1.22–3.6 mmoles) of methanol-C^{14}; then vigorous boiling is resumed. About 30 minutes is required to complete the reaction. The air stream is shut off, and the frozen distillate is thawed; yield 77.5–81.2%, 79.1% average of 7 runs (Note 18).

METHOD V

$$C^*H_3OH \xrightarrow{CH_3COCl} \underset{(a)}{CH_3COOC^*H_3} \xrightarrow{Cl_2} \underset{(b)}{CH_3COOC^*H_2Cl} \xrightarrow{H_2O} \underset{(c)}{HC^*HO}$$

A. R. Jones and W. J. Skraba, J. Am. Chem. Soc., *74*, 2437 (1952); Science, *110*, 332 (1949).

A. Procedure (Note 19)

(a) *Methyl-C^{14} Acetate.* Methanol-C^{14} (0.322 g., 10.4 mmoles) and 0.816 g. (10.4 mmoles) of acetyl chloride are distilled under high vacuum into the liquid nitrogen-cooled nipple of a 1-l. bulb, which then is closed off from the manifold. The mixture is heated to 40–50° for 45 minutes, then refrozen into the nipple. The frozen mixture is warmed in a Dry Ice-bath, and the flask is evacuated to 0.1 μ to remove much of the hydrogen chloride formed (Note 20).

(b) *Chloromethyl-C^{14} Acetate.* To the crude methyl-C^{14} acetate, frozen in the nipple of the reaction bulb with liquid nitrogen, is added 9.6 mmoles of chlorine (Note 21). The bulb is isolated from the manifold, and the contents are thoroughly mixed by several cycles of thawing and

freezing. At the completion of reaction, when all trace of chlorine color has disappeared (1–2 hours), hydrogen chloride again is removed as described above (Note 22).

(c) *Formaldehyde-C^{14}*. The chloromethyl-C^{14} acetate is vacuum-distilled into a 25-ml. flask equipped with a pressure-stopcock and containing 3 ml. of water frozen by liquid nitrogen. The flask is closed off, removed from the manifold and heated in a boiling water-bath for 30 minutes. The mixture is cooled, the stopcock is removed with a file, and the contents of the flask are transferred into a 20-ml. pear-shaped flask by washing with 5 ml. of 37% formalin (60 mmoles). The pH is adjusted with solid potassium hydroxide and glacial acetic acid to just acid to phenolphthalein; then the mixture is distilled to dryness into an ice-cooled receiver (Note 23). The yield of neutral formalin solution is 9.027 g., and the radiochemical yield is 60.5% based on methanol (Note 24).

(d) *Paraformaldehyde-C^{14}* is prepared by treating chloromethyl-C^{14} acetate with sufficient commercial formalin solution to furnish the water for hydrolysis. Evaporation under high vacuum leaves a residue of dry formaldehyde-C^{14} polymer.

METHOD VI

$$(C_6H_5)_3CBr \xrightarrow{C^*H_3OH} \underset{(a)}{(C_6H_5)_3COC^*H_3} \xrightarrow[\Delta]{} \underset{(b)}{HC^*HO} + C^*O + C^*H_3OH + (C_6H_5)_3CH$$

A. R. Van Dyken, Atomic Energy Commission Report, ANL-4438; Nuc. Sci. Abstracts, 5, 2748 (1951).

A. Procedure (Note 25)

(a) *Methyl-C^{14} Triphenylmethyl Ether* (Note 26). To a solution of 2.4746 g. (7.68 mmoles) of triphenylmethyl bromide (m.p. 151–152°), 15 ml. of benzene and 1.0 g. of dry pyridine, frozen with liquid nitrogen, is added 0.2461 g. (7.68 mmoles) of methanol-C^{14} by vacuum distillation. The flask is isolated from the vacuum line, and the mixture is stirred at room temperature for 1 hour with an induction stirrer. The mixture is distilled to dryness under reduced pressure, and the residue is treated with 10 ml. of water and extracted with 10 ml. of benzene. The extract is washed with 5 ml. of water and passed through a column of alumina (1 × 30 cm.) by washing with 25 ml. of benzene. The first 15 ml. of filtrate, upon evaporation of the solvent, yields 1.9225 g. of product (91.3%), m.p. 82–83°

(b) *Formaldehyde-C^{14}* (Note 27). The pyrolysis apparatus consists of a U-tube (with a gas inlet) which is attached to a train consisting of: two bubble traps containing 10 ml. of water at 0°, a U-tube at −195°, a U-tube containing iodine pentoxide at 110–120°, a U-tube at −195°, a quartz tube containing copper oxide at 900°, a U-tube at −78°, a U-tube at −195° and a bubbler tube (Note 28). Methyl-C^{14} triphenylmethyl ether (0.5428 g., 2.00 mmoles) is heated at 390–400° (salt-bath) in a slow stream of helium for at least 90 minutes; then the gas flow is continued for 45 minutes. The yield is 65–70%, which, with the methanol-C^{14} and the carbon-C^{14} monoxide formed, accounts for 95% of the original C^{14}-methoxyl group (Note 29). The molar specific activities of all compounds agree with that of the starting material, and the method appears to be satisfactory for preparing the anhydrous product.

B. Notes

1. Reaction times of 15 and 45 minutes at 0° diminish the yield to 57% and 59%; the yield falls to 43% when one-half molar quantities of hydride are used. Nystrom has reported the formation of up to 25% formaldehyde resulting from the absorption of carbon dioxide by lithium aluminum hydride in ether; see Note 26 and methanol-C^{14}, Method I. Lithium aluminum hydride solution is assayed by measuring the hydrogen liberated with glycol.

Another worker[1] has obtained maximum yields of 55% (45 minutes, −5°), determined gravimetrically as the 2,4-dinitrophenylhydrazone.[1a]

2. The procedure is an adaptation of the general method of Weygand[1b] for preparing aldehydes from carboxylic acids by reducing the *N*-methylanilides with lithium aluminum hydride.[2]

3. The procedure is a modification of the method of Fieser and Jones.[3] The yield is 90% when the free acid is heated with the anilide in toluene.

4. Tetrahydrofuran is purified by standing over alkali for 2 weeks. and by being distilled from sodium and redistilled from lithium aluminum hydride. The reagent solution is standardized manometrically by decomposing with glycol in tetrahydrofuran.

5. With reaction times of 1–2 hours, the yield for the reduction step is 85–95%, determined by forming the 2,4-dinitrophenylhydrazone (m.p. 161°) or the formal dimedon (m.p. 189°).

6. As the result of about sixty preliminary experiments with the apparatus described, it was established graphically that the optimum temperature is 687° and a fluctuation of 10° decreases the yield less than 1%. Shortening the reaction time from 7 hours to 1 hour does not measureably affect the yield; further reducing it to 15 minutes reduces the yield by about 1%.

7. A rotameter is used to indicate the rate of flow in the feed line.

8. The condensate contains 15.28 g. of formaldehyde as a 25% aqueous solution, 0.5 g. of methanol and 2 mg. of formic acid; the aqueous washings from the scrubber contain an additional 0.28 g. of formaldehyde. The exhaust gas contains 5% carbon dioxide and 11.4% hydrogen.

9. The catalyst attains optimum efficiency after a given time.

10. The yield is based on past performance of the apparatus.

11. The procedure is a modification of the method of Meharg and Adkins.[4]

12. Compressed air, controlled by needle valve, is passed in turn through a safety trap, a gas washing bottle filled with concentrated sulfuric acid, a tower of calcium chloride and another containing potassium hydroxide pellets. The pressure is maintained at the desired constant value by adjusting the head of mercury in a pressure regulator.

13. The heating coil consists of 6.5 feet of No. 30 nichrome wire wrapped over a layer of asbestos paper and is controlled with a variable transformer.

14. A 50-ml. round-bottomed flask containing 25 ml. of water serves as the generator. Both side arms are heated at approximately 100° by a second electrical winding.

15. A diagram of a similar system is presented by Arnstein.

16. Kögl[5] has reported a temperature of 500–550° to be satisfactory.

17. Kögl[5] has reported an 80% yield of formaldehyde-C^{14} by employing a temperature of 395–400° and an air flow of 120–150 ml. per minute. Stutz[6] has reported the isotopic preparation in 70–85% yield by operating with a batch of catalyst[4] at 255–260° and 100–150 ml. per minute; a Hopcalite catalyst[7] at 50–55° was introduced to oxidize the carbon-C^{14} monoxide for recovery as barium carbonate-C^{14}. Arnstein found 350–400° and 120–150 ml./min. to be the optimum conditions for his apparatus.

18. The yield is determined by weighing the insoluble derivative formed with dimedon or with 4-hydroxycoumarin; see 3,3′-methylene-C^{14}-bis(4-hydroxycoumarin). A micro-determination of formaldehyde by Schryver's method is described by Dowse.[8]

A standard formaldehyde solution, made by diluting 10 ml. of redistilled formalin to 1 l., was assayed by several methods. The results (mg./ml.) are the average of 3–5 determinations: a) precipitation of the dimedon derivative,[9] 2.865; b) precipitation of the 4-hydroxycoumarin derivative, 3.121; c) oxidation of formaldehyde with hydrogen peroxide,[10] 3.103; d) reduction of silver nitrate with formaldehyde in the presence of aqueous triethylamine, 2.923.

When the 4-hydroxycoumarin derivative is precipitated in the presence of excess formaldehyde, the yield drops to 96.5%.

19. The procedure is a modification of the method of Henry[11] and Michael.[12] The isotopic method has been improved by Iliceto[12a] through

the use of trichloroacetyl chloride, the advantages being the higher boiling point of the ester (methyl-C^{14} acetate, 57°; methyl-C^{14} trichloroacetate, 154°) and the greater ease of determining the chlorination endpoint, with the attendant higher yield of formaldehyde-C^{14} solution (75–78%). An apparatus diagram and detailed directions at the 15.4-mmole scale are given by Iliceto.[12a]

20. The yield is not improved by acetylating under reflux at atmospheric pressure, and the yield is definitely decreased when the acetylation is conducted in the presence of one equivalent of pyridine.

Methyl-C^{14} trichloroacetate is formed in 99–100% yield by stirring equivalent amounts of the reactants at room temperature for 2 hours, and contains 2.5–3% of dissolved hydrogen chloride.[12a]

21. The chlorine is purified by pumping off any gas not condensed by liquid nitrogen. The chlorine is measured in a known volume by determining the pressure with a manometer in which the mercury is protected by a layer of sulfuric acid. The over-all yield of formaldehyde is best when chlorine is not present in excess.

The required amount of chlorine may be measured[12a] by determining the volume occupied at −75° in a calibrated capillary, any excess being boiled off.

22. The yield is not affected by varying the temperature from 20–60°, nor by ultraviolet irradiation. After a number of runs, the reaction bulb may contain a small amount of white, nonvolatile residue which does adversely affect the yield of formaldehyde. In attempts to avoid the competing chlorination of the methyl group of the acetyl moiety by employing the methyl esters of various halo-acetic (see Note 19), oxalic, benzoic, *p*-toluenesulfonic and chlorocarbonic acids, the yield of formaldehyde was very poor. Bromination gave very poor results.

The yield of chloromethyl-C^{14} trichloroacetate[12a] apparently reaches an optimum at 78% when an equimolar mixture of the ester and chlorine is stirred at 25° for 2.1 hours under illumination from an arc lamp. Optimum conditions are arrived at by hydrolyzing the chloroester with 2 *N* sodium hydroxide at room temperature and titrating the formaldehyde with 0.02 *N* iodine according to Romijn. The reduction in yield is due to formation of the di- and tri-chloromethyl esters.

23. Jones and Skraba present a diagram showing the receiver as part of an aliquoting apparatus.

24. It has been shown in another preparation that no isotopic dilution occurs. The hydrolysis of chloromethyl acetate is quantitative. The formaldehyde is assayed as the dimedon derivative, prepared as follows. To a solution of 0.078 g. of formaldehyde-C^{14} is added a solution of 0.40 g. of dimedon in 100 ml. of water. After standing 24 hours at room temperature, the precipitate is collected, washed with water and dried to give 0.159 g. of formal-C^{14} dimedon.

The hydrolysis of chloromethyl-C^{14} trichloroacetate at 5–10° with 3 *N* sodium hydroxide is quantitative. The resultant solution, containing sodium chloride, sodium trichloroacetate and formaldehyde-C^{14}, is suitable for many purposes without purification.

25. The method is based on the studies of Norris[13,14] on the pyrolytic decomposition of aliphatic trityl ethers. A number of preparative methods were investigated for the purpose of preparing isotopic anhydrous formaldehyde at the millimole level: a) reduction of carbon dioxide with a stoichiometric amount of ethereal lithium aluminum hydride at −78° (yield 25%), b) bromination of *o*-hydroxybenzyl alcohol at 40–50° (yield 71%, as an aqueous solution), c) pyrolysis of triphenylmethanol at 380–400° (yield 45–50%), d) pyrolysis of 1,3-dioxo-2-isoindolenemethanol at 135–190° (yield 92%), e) pyrolysis of 3,6-dichloro-9-carbazolemethanol at 150–200° (yield 90%), f) pyrolysis of 9,10-dihydro-9,10-dimethoxy-9,10-diphenylanthracene at 380–390° (yield 21.4%), and g) pyrolysis of methyl tri-4-biphenylylmethyl ether at 380–390° (yield 16.8%).

26. The procedure is a modification of the method of Norris and Young;[13] also see Swain.[15]

27. The procedure is based on a study of the effects of time, temperature and catalyst on the pyrolysis.

28. The hot iodine pentoxide oxidizes the carbon-C^{14} monoxide to carbon-C^{14} dioxide. Any noncondensable, combustible gases are oxidized to carbon-C^{14} dioxide by the hot copper oxide.

29. When the heating period is 80 minutes at 384–394°, the two aqueous traps contain 0.80 mmole of formaldehyde-C^{14} (40% yield) mixed with 0.707 mmole of methanol-C^{14} (35.4% yield). The total methanol and formaldehyde are determined by permanganate oxidation[16] of an aliquot, while another aliquot is assayed for formaldehyde by the dimedon method.[17] The yield of carbon-C^{14} monoxide is 0.367 mmole (18.4% yield), and the residue consists of 0.4858 g. of benzene-soluble material, including 2.0% of unchanged starting material. Directions are given by Van Dyken for assaying the residue for starting material by applying the principles of isotope dilution technique and selective adsorption on alumina.

C. Other Preparations

Formaldehyde-C^{14} has been prepared by Siegel[18] by air oxidation of methanol-C^{14} over a vanadium-molybdenum oxide catalyst. The vapors were passed over calcium chloride to remove water (preventing polymerization on the walls) and unreacted starting material, then were trapped in water containing a trace of methanol.

Formaldehyde-C^{14} has been prepared in a similar manner by Elwyn,[19] by passing the vapors of 3.0 g. of methanol-C^{14} in an air stream over a vana-

dium-molybdenum catalyst at 320°. The product was collected in 5 ml. of water in two traps; yield 65–70%. A diagram of the apparatus and directions for preparing the catalyst are given.

Formaldehyde-C^{14} has been prepared in 40–60% yield by Heidelberger,[20] by passing an air stream containing 20–25 mmoles of wet methanol-C^{14} over a copper catalyst at 600°, and trapping the products in a bubbler containing 1 ml. of water with a trace of methanol. Tolbert and Christenson[21] have reported a 50–60% yield by a similar procedure.

The preparation of formaldehyde-C^{14} by pyrolysis of 1,1,1-triphenylethanol-2-C^{14}, under the influence of catalysts, has been reported by Pichat[22] to give less than 10% yields.

Formaldehyde-C^{14} has been prepared[23] by reduction of formic-C^{14} acid with magnesium and hydrochloric acid. The hydrazone, prepared by treatment with excess of 2,4-dinitrophenylhydrazine, was extracted with benzene, evaporated and hydrolyzed by distillation from 5 *N* sulfuric acid with passage of the product through a column of Dowex-1 (chloride form).

[1]D. G. Ott, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, private communication.

[1a]H. A. Iddles and C. E. Jackson, Ind. Eng. Chem., Anal. Ed., *6*, 454 (1934).

[1b]F. Weygand, G. Eberhart, H. Linden, F. Schäfer, and I. Eigen, Angew. Chem., *65*, 525 (1953).

[2]*Organic Reactions*, Vol. 6, Wiley, New York, 1951, p. 469.

[3]*Organic Syntheses*, Coll. Vol. III, Wiley, New York, 1955, p. 590.

[4]V. E. Meharg and H. Adkins, U.S. 1,913.405; Chem. Abstracts, *27*, 4252 (1933).

[5]K. Kögl and A. M. Municio, Hoppe-Seyler's Z. physiol. Chem., *300*, 17 (1953).

[6]R. E. Stutz, D. E. Atkinson and S. A. Gordon, Atomic Energy Commission Report, ANL-4710; Nuc. Sci. Abstracts, *6*, 164 (1952).

[7]W. E. Kuentzel, J. Am. Chem. Soc.. *52*, 445 (1930).

[8]C. M. Dowse and J. A. Saunders, Biochem. J., *60*, XXI (1955).

[9]F. Wild, *Characterization of Organic Compounds*, Cambridge Univ. Press, London, 1947, p. 135; J. H. Yoe and L. C. Reid, Ind. Eng. Chem., Anal. Ed., *13*, 238 (1941).

[10]O. Blank and H. Finkenbeiner, Ber., *31*, 2979 (1898).

[11]L. Henry, Ber., *6*, 739 (1873).

[12]A. Michael, Am. Chem. J., *1*, 418 (1879).

[12a]A. Iliceto, Ann. chim. (Rome), *43*, 638 (1953).

[13]J. F. Norris and R. C. Young, J. Am. Chem. Soc., *52*, 753 (1930).

[14]J. F. Norris and A. Cresswell, *ibid.*, *55*, 4946 (1933).

[15]C. G. Swain, *ibid.*, *70*, 1119 (1948).

[16]R. Oesper, *Newer Methods of Volumetric Analysis*, Van Nostrand, New York, 1938, pp. 55–66.

[17]W. Weinberger, Ind. Eng. Chem., Anal. Ed., *3*, 365 (1931).

[18]I. Siegel and J. Lafoye, Proc. Soc. Exptl. Biol. Med., *74*, 620 (1950).

[19]D. Elwyn and D. B. Sprinson, J. Biol. Chem., *184*, 465 (1950).

[20]C. Heidelberger, J. Biol. Chem., *179*, 139 (1949).

[21]M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 166.

[22]L. Pichat and M. Audinot, Bull. soc. chim. France, *19*, 466 (1952).

[23]F. C. Charalampous and G. C. Mueller, J. Biol. Chem., *201*, 161 (1953); W. M. Grant, Anal. Chem., *20*, 267 (1948).

ACETALDEHYDE-C_2^*

METHOD I

$$HC^*{\equiv}C^*H \xrightarrow[HgSO_4]{H_2O} C^*H_3C^*HO$$

R. D. Cramer and G. B. Kistiakowsky, J. Biol. Chem., *137*, 549 (1941).

A. Procedure

Acetaldehyde-C_2^{11}. A mixture of about 0.6 mmole of acetylene-C_2^{11} and 10 ml. of an aqueous catalyst solution,[1] containing 0.2 g. of mercuric sulfate and 0.6 g. of sulfuric acid, is heated for five minutes at 100° in a sealed tube. The product is distilled from the mixture in a current of air; yield 75% or more.

METHOD II

$$C^\circ H_3C^*H_2OH \xrightarrow{Na_2Cr_2O_7} C^\circ H_3C^*HO$$

G. Ehrensvard, L. Reio, E. Saluste, and R. Stjernholm, J. Biol. Chem., *189*, 93 (1951).

A. Procedure

Acetaldehyde-2-C^{13}-1-C^{14}. Ethanol-2-C^{13}-1-C^{14} is oxidized by an adaptation of the method of Wertheim,[2] which follows.

The apparatus consists of a 2-l. round-bottomed flask equipped with a dropping funnel leading nearly to the bottom of the flask, and a reflux condenser (12 mm. × 80 cm., or longer) cooled with water at 5° and containing a thermometer suspended near the outlet, which is connected to two gas-absorption bottles containing 150 ml. and 125 ml. of absolute ether, respectively (Note 1). Mixing is achieved by bubbling carbon dioxide through the reaction mixture at such a rate as to keep the contents of the flask in active motion. To 25 ml. of stirred ethanol, heated to boiling with a small flame which is then lowered, is added slowly an acid dichromate mixture (Note 2) at such a rate that the temperature of the effluent gas lies between 20° and 25° (about 20 min.). The mixture then is boiled for ten minutes; yield 70–72%, determined as the aldehyde-ammonia (Note 1). A degradation with hypoiodite is described.

B. Notes

1. The traps are arranged for isolation of the product as the crystalline aldehyde-ammonia, which is formed by passing dry ammonia gas into the ether solution and setting aside for 3–4 hours to crystallize.

2. The oxidizing mixture is prepared by adding 115 g. of nitric acid (d. 1.42) to 60 g. of sodium dichromate, followed by 220 ml. of water and 20 g. of sulfuric acid (d. 1.84). If 60 g. of potassium dichromate is used, 300 ml. of water is required.

3. The authors[3] present a diagram of the preparative apparatus. The degradation with potassium hypoiodite forms formic-C^{14} acid and iodoform-C^{14}, respectively. The vapor pressure of the acetaldehydes is in agreement with that of an authentic pure sample.

C. Other Preparations

By a procedure similar to Method II are prepared[3] acetaldehyde-1-C^{14} and acetaldehyde-2-C^{14}, in 70% yield, from ethanol-1-C^{14} and ethanol-2-C^{14}, respectively (Note 3).

Both acetaldehyde-C_2^{13} and acetaldehyde-C_2^{14} have been prepared by Gurin and co-workers,[4] employing Method I. Each product was oxidized with excess silver oxide and isolated as the sodium acetate-C_2^*; yield 40% based on barium carbonate.

Acetaldehyde-C_2^{13} has been prepared by Lifson,[5] employing a modification of Method I. The acetylene-C_2^{13}-hydrogen mixture, formed by hydrolysis of the carbide-C_2^{13} prepared from 50 mmoles of barium carbonate-C^{13}, was collected over water in a 3-l. flask, then passed through a sintered glass disc into 150 ml. of boiling catalyst solution. The product was collected in 50 ml. of 5% sodium bisulfite, the outgoing gas being recycled through the catalyzing solution. The acetaldehyde-C_2^{13} was distilled from the bisulfite solution, after addition of calcium carbonate.

Acetaldehyde-C_2^{13} has been prepared by Wood,[6] by a modification of Method I. Acetylene-C_2^{13} was swept with nitrogen through a solution of mercuric chloride at 100°, then into a trap containing bisulfite solution. The product was released by treatment with dipotassium phosphate, distilled and oxidized to acetic-C_2^{14} acid with dichromate.

The preparation of acetaldehyde-C_2^{14} by Method I has been reported by Mizuhara[7] and described in detail with apparatus diagrams by Reitz[8] (yield 62% as the dimedon based on barium carbonate).

Isagulyants[9] has reported the preparation of acetaldehyde-C_2^{14} in 75–85% yield by Method I. To a warm solution of 5 ml. of concentrated sulfuric acid in 11 ml. of water is added 0.5 g. of mercuric oxide. The apparatus is evacuated to 1 mm., acetylene-C_2^{14} is passed in at 15–18° (the

yield drops at 30°), and the product is steam-distilled through a water-cooled reflux condenser into a receiver chilled with liquid nitrogen.

[1]R. Scharf, Z. physik, u. chem. Unterricht, *50*, 239 (1937); Chem. Abstracts, *32*, 2084 (1938).

[2]E. Wertheim, J. Am. Chem. Soc., *44*, 2658 (1922).

[3]M. B. Neyman and G. I. Feklisov, Doklady Akad. Nauk S.S.S.R., *87*, 605 (1952); Chem. Abstracts, *48*, 114 (1954); AEC-tr-1828; Nuc. Sci. Abstracts, *7*, 4095 (1953); G. I. Feklisov, Izvest. Akad. Nauk, S.S.S.R., Otdel. Khim. Nauk, *1953*, 587; Chem. Abstracts, *48*, 12666 (1954).

[4]W. Sakami, W. E. Evans and S. Gurin, J. Am. Chem. Soc., *69*, 1110 (1947); S. Gurin and A. M. Delluva, J. Biol. Chem., *170*, 545 (1947).

[5]N. Lifson, V. Lorber, W. Sakami and H. G. Wood, J. Biol. Chem., *176*, 1263 (1948).

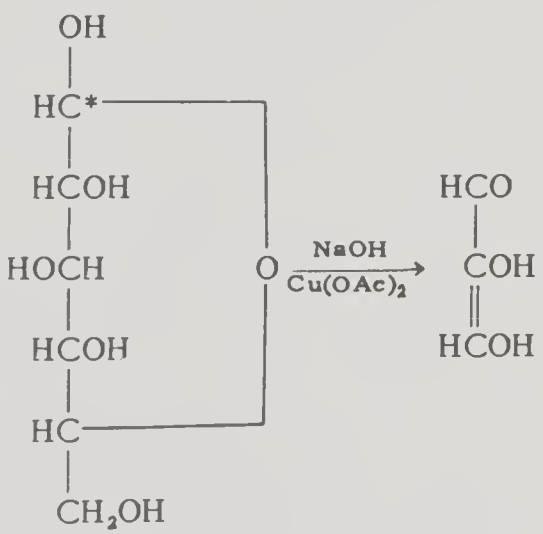
[6]H. G. Wood, J. Biol. Chem., *194*, 905 (1952).

[7]S. Mizuhara and P. Handler, J. Am. Chem. Soc., *76*, 571 (1954).

[8]H. C. Reitz, F. J. Carleton and J. E. Christian, J. Am. Pharm. Assoc., Sci. Ed., *42*, 85 (1953).

[9]G. V. Isagulyants and O. A. Golvina, Doklady Akad. Nauk S.S.S.R., *93*, 659 (1953); Chem. Abstracts, *49*, 1543 (1955).

C^{14}-HYDROXYPYRUVALDEHYDE
(C^{14}-Triose Reductone)



H. F. Bauer and C. Teed, Can. J. Chem., *33*, 1824 (1955).

A. Procedure (Note 1)

To a solution of 25 g. of D-glucose-1-C^{14} in 375 ml. of water is added 13.5 g. of lead acetate, 0.003 g. of potassium cyanide and 0.050 g. of copper acetate (Note 2). The mixture is heated to 92° in a stream of ni-

trogen, then treated with 8.5 g. of sodium hydroxide in 25 ml. of water, shaken for 2–3 minutes, acidified with 4 ml. of glacial acetic acid and cooled quickly. The lead salt is collected by centrifuging, washed with water, acetone and ether, and dried under vacuum; yield 12.5 g. (Note 3).

To a suspension of the C^{14}-lead salt in 50 ml. of anhydrous acetone is added the calculated amount (4.5 ml.) of 85% phosphoric acid. The mixture is shaken for 30 minutes and filtered, and the filtrate is concentrated under vacuum. The residue is cooled to −15°, and the crystalline product is collected on a filter and sublimed at 5 μ and 60–80° (bath); yield 0.85 g., m.p. 153° (Note 4).

B. Notes

1. The isotopic distribution in C^{14}-reductone is uncertain. The procedure is essentially that of Euler.[1]

2. Copper functions as a catalyst in this reaction.[2]

3. The lead assay is 58.8%. There is no glycerol in the reaction mixture.

4. The activity of the product is 10% greater than that of the starting material (38.9 μC/mmole CO_2), indicating that carbons 1–3 of D-glucose contribute: $\frac{38.9 \times 1.1}{2 \times 38.9}\,100 = 55.1\%$ of the yield of reductone, the remainder being contributed by carbon 4–6.

The ultraviolet absorption spectrum of triose-reductone is described by Euler.[3,2,1] Pure reductone darkens at 150–170°, melts at 200–220° (dec.), and may be titrated[1] with alkali and phenolphthalein.

[1]H. v. Euler and C. Martius, Svensk Kem. Tids., *45*, 73 (1933); Chem. Abstracts, *27*, 3452 (1933); H. v. Euler and H. Hasselquist, Arkiv. Kemi, Mineral. Geol., *26A*, No. 25 (1949); Chem. Abstracts, *43*, 7903 (1949).

[2]H. v. Euler, H. Hasselquist and G. Hanshoff, Arkiv. Kemi, *6*, 471 (1953); Chem. Abstracts, *48*, 6324 (1954).

[3]H. v. Euler, H. Hasselquist and G. Hanshoff, Z. Naturforsch., *8b*, 636 (1953).

CROTONALDEHYDE-C_4^{14}

$$C^*H_3C^*HO \xrightarrow{KOH} \underset{\displaystyle OH}{\underset{|}{C^*H_3C^*HC^*H_2C^*HO}} \xrightarrow{I_2} C^*H_3C^*H{=}C^*HC^*HO$$

(a) (b)

R. O. Brady, J. Rabinowitz, J. Van Baalen and S. Gurin, J. Biol. Chem., *193*, 137 (1951).

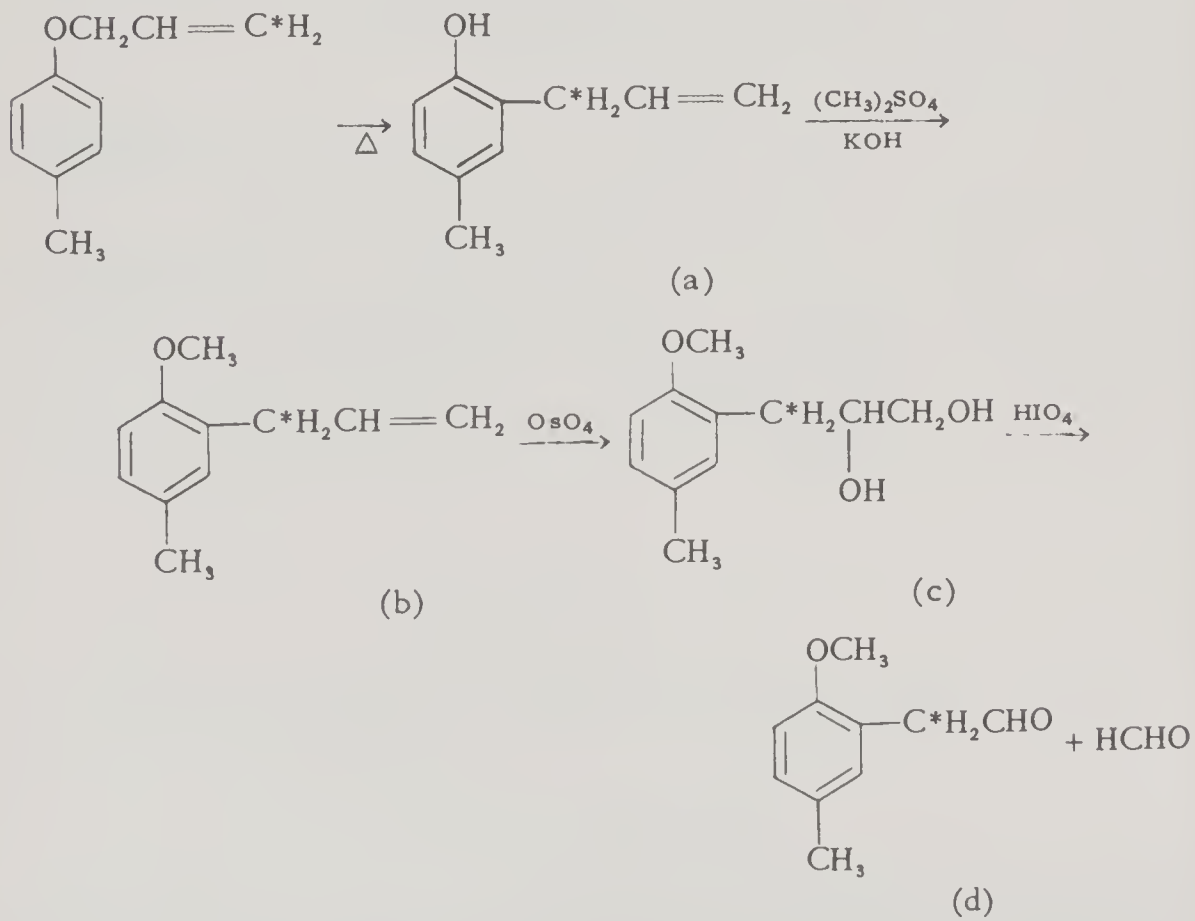
Procedure

(a) *3-Hydroxybutyraldehyde-C_4^{14}*, (*Acetaldol-C_4^{14}*). Acetaldehyde-C_2^{14} is converted to aldol in dilute aqueous potassium hydroxide solution according to the procedure of Hammarsten.[1] The Cannizzaro reaction is much slower than the aldol formation, but becomes more pronounced with higher concentrations of alkali than 0.5 *N* potassium hydroxide. The reaction is practically complete by the end of 3 hours. The product is extracted into ether and isolated by several fractional distillations of 9–10 mm. with a bath temperature of 60–90°; yield 34%.

(b) *Crotonaldehyde-C_4^{14}*, (*3-Methylacrolein-C_4^{14}*). The dehydration of acetaldol-C_4^{14} is readily effected by slow distillation through a short column in the presence of catalytic amounts of iodine.[2] The distillate is dried and fractionated, and the portion distilling at 90–105° is collected; yield 67%.

[1]H. Hammarsten, Ann., *421*, 293 (1920).
[2]H. Hibbert, J. Am. Chem. Soc., *37*, 1748 (1915).

(6-METHOXY-*m*-TOLYL)ACETALDEHYDE-2-C^{14}



H. Schmid and K. Schmid, Helv. Chim. Acta, *35*, 1879 (1952).

A. Procedure (Note 1)

(a) *2-Allyl-1-C*^{14}-p-*cresol* (Note 2). Allyl-3-C^{14} *p*-tolyl ether (0.549 g.) is sealed in a small bomb tube at −80° under high vacuum, then heated for 13 hours at 200°. The reaction mixture is distilled, and the crude product is collected between 115–120° at 11 mm. (Note 3). After redistillation, the yield is 0.486 g. (85%), n_D^{20} 1.5353, and the molar specific activity is the same as that of the starting material. Degradation studies (see below) show that there is inversion but no isotopic rearrangement.

(b) *4-Methyl-o-estragole-α-C*^{14}, (*2-Allyl-1-C*^{14}*-4-methylanisole*). To a stirred mixture of 0.397 g. of 2-allyl-1-C^{14}-*p*-cresol, 0.300 g. of potassium hydroxide, and 2.5 ml. of water at 100° is added 0.340 g. of methyl sulfate. In the course of the next hour an additional amount of alkali, water and methyl sulfate is added. After 1.5 hours, the mixture is stored for 20 hours at 20°, then extracted with ether. The ether solution is washed with 25% potassium hydroxide solution and water, dried, and concentrated by distillation through a column. The product is distilled at 10 mm. with an air-bath at 95–100°. The yield is nearly quantitative.

(c) *3-(6-Methoxy-m-tolyl)-1,2-propanediol-3-C*^{14}. A solution of 0.216 g. of 4-methyl-o-estragole-α-C^{14} in 8.5 ml. of absolute ether is treated with 0.335 g. of osmium tetroxide in 16 ml. of ether and 0.27 ml. of anhydrous pyridine, then left overnight at 20°. The solvent is evaporated under reduced pressure, and the residue is mixed with 5 ml. of chloroform, 0.105 g. of sodium hydroxide, 0.480 g. of mannitol and 15 ml. of water, then stirred until the chloroform layer is colorless (1 hour). The brown, crystallizing oil, obtained by extraction with ether, is washed with petroleum ether; yield 0.153 g. (60%). The melting point is 56–57° after two recrystallizations from acetone-petroleum ether, and the molar specific activity is the same as that of the starting material.

(d) (*6-Methoxy-m-tolyl*)*acetaldehyde-2-C*^{14}. A mixture of 0.064 g. of the diol in 5 ml. of water and 0.069 g. of periodic acid in 1 ml. of water, neutralized with sodium bicarbonate, is stored overnight at 20°. The mixture is extracted several times with ether, and the extract is washed with water and evaporated to dryness. The product is converted to the semicarbazone for assay (Note 4) and repeatedly crystallized from methanol. The yield is 0.038 g. (52%), m.p. 169–170°, and the molar specific activity is the same as that of the starting material (Note 5).

B. Notes

1. The synthesis is a study of the mechanism of the classical thermal *ortho* Claisen rearrangement[1] of the allyl ether of a phenol (or enol) to the

isomeric C-allyl compound (with α-γ inversion of the allyl group). For the *para* rearrangement (without inversion), see 4-allyl-3-C^{14}-2,6-xylenol.

2. The procedure is a modification of the method of Kincaid and Tarbell,[2] which is reported to give 97.7% rearrangement. The presence of oxygen does not appreciably affect the reaction, but does develop a color. An equimolecular mixture of allyl-3-C^{14} *p*-tolyl ether and 4′-allyloxyacetophenone, subjected to the conditions of the Claisen rearrangement, gave nonisotopic 3′-allyl-4′-hydroxyacetophenone (52% yield), indicating that the reaction is strictly intramolecular[3] because of the absence of cross-products.

3. The phenol is often isolated by extraction with 10–20% potassium hydroxide, or with Claisen's alkali. The latter is prepared by diluting a solution of 350 g. of potassium hydroxide and 250 ml. of water to 1 liter with methanol.

4. The residue is dissolved in a few drops of methanol, then treated with a filtered solution of 39 mg. of semicarbazide hydrochloride and 52 mg. of sodium acetate in 2 ml. of water and heated for 1 hour at 90–100°. The mixture is cooled, and the semicarbazone is collected and crystallized from methanol.

5. From the aqueous phase is obtained nonisotopic formaldehyde, as the dimedon derivative (73%), m.p. 189.5–190.5°. See formaldehyde-C^{14}, Method III, Note 6. Ryan[4] has oxidized the aldehyde groups with silver oxide and isolated the corresponding acetic acids.

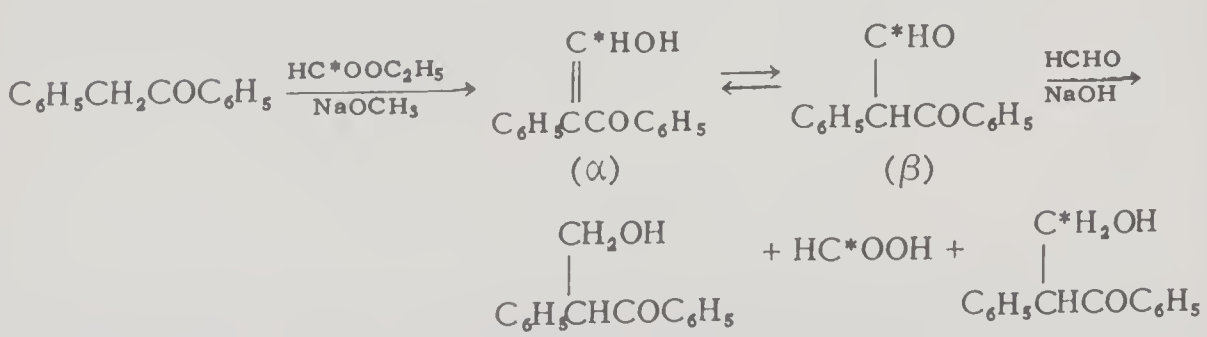
[1]D. S. Tarbell, Chem. Rev., *27*, 495 (1940); *Organic Reactions*, Vol. 2, Wiley, New York, 1944, Chapter 1.

[2]J. F. Kincaid and D. S. Tarbell, J. Am. Chem. Soc., *61*, 3085 (1939).

[3]H. Schmid and K. Schmid, Helv. Chim. Acta, *35*, 1879 (1952).

[4]J. P. Ryan and P. R. O'Connor, J. Am. Chem. Soc., *74*, 5866 (1952).

BENZOYLPHENYLACETALDEHYDE-1-C^{14}
(3-Hydroxy-2-phenylacrylophenone-3-C^{14})
(Formyl-C^{14}-deoxybenzoin)



J. G. Burr, Jr., J. Am. Chem. Soc., *73*, 5170 (1951).

A. Procedure (Note 1)

A solution containing 15.70 g. (80 mmoles) of deoxybenzoin (Note 2) and 6.86 ml. (85.6 mmoles) of ethyl formate-C^{14} in 50 ml. of absolute ether is stirred with 4.86 g. (90 mmoles) of sodium methoxide in a nitrogen atmosphere at room temperature for 60 hours (Note 3). The mixture is hydrolyzed with water, and the ether layer is extracted several times with dilute sodium hydroxide. The aqueous solutions are washed with ether, chilled and cautiously acidified. The yellow product is collected and air-dried (Note 4); yield 9.53 g. (53%), m.p. 80–82° (Note 5).

B. Notes

1. Formyldeoxybenzoin, b.p. 183° (14 mm.), occurs in two desmotropic forms:[1] the yellow enol-keto, α-form, melts at 76–80°, while the colorless aldo-enol, β-form, melts at 112–113°. Burr[2] has reported a spontaneous dehydration of the compound with dimerization of the 2-phenylacrylophenone formed.

2. The deoxybenzoin is purified by digestion with dilute sodium hydroxide solution and recrystallization from methanol.

3. The yield drops to 31% (92% purity) when the synthesis is carried out with equimolecular amounts of reactants in alcohol solution.

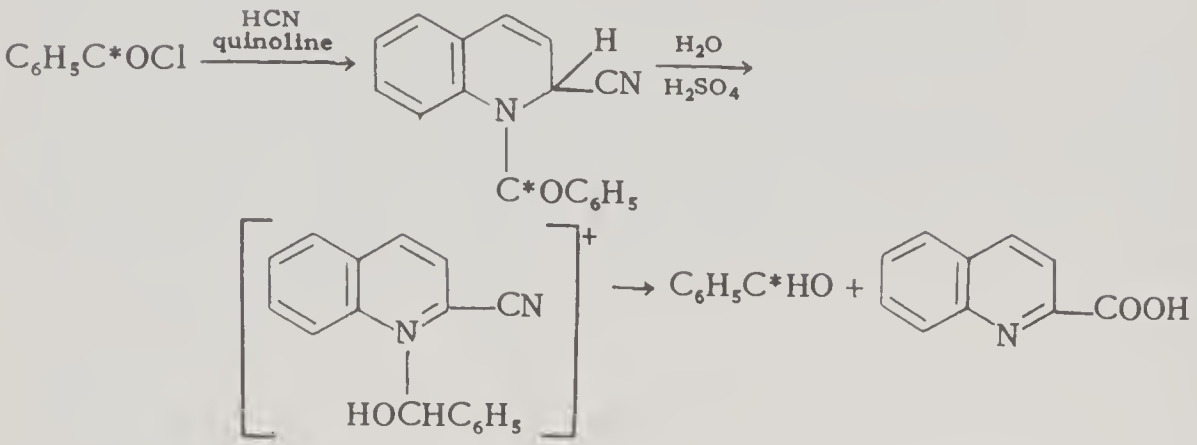
4. Benzoylation,[1] in dilute alkaline solution with benzoyl chloride, forms 3-oxo-2,3-diphenylpropenyl-1-C^{14} benzoate, m.p. 106–107°, which is crystallized from dilute alcohol. The molar specific activity is unchanged.

5. In the presence of excess formaldehyde in basic media, aliphatic aldehydes with one α-hydrogen atom undergo the aldol condensation, but the β-hydroxyaldehyde formed apparently does not then undergo the usual crossed Cannizzaro reaction,[2] leading to the formation of a bis(hydroxymethyl)alkane and formic acid, unless there is a high electron density at the α-carbon.[3,4] In cases where substituent groups have decreased the electron density, the compound instead undergoes displacement of the formyl group (see 9-fluorenecarboxaldehyde-C^{14}). In the present instance, 3-hydroxy-2-phenylpropiophenone-3-C^{14} (or its decomposition compounds) is formed containing 3–10% of the molar specific activity of the starting material, apparently through a second path involving direct reduction of the aldehyde. The formic-C^{14} acid is precipitated[5] for assay as *S*-benzylthiuronium formate-C^{14}, m.p. 146–147°; radiochemical yield 57%.

[1] W. Wislicenus and A. Ruthing, Ann., 379, 229 (1911).
[2] *Organic Reactions*, Vol. II, Wiley, New York, 1944, p. 100.
[3] J. G. Burr, Jr., J. Am. Chem. Soc., 73, 5170 (1951).
[4] J. B. Conant and G. W. Wheland, J. Am. Chem. Soc., 54, 1215 (1932).
[5] J. J. Donleavy, J. Am. Chem. Soc., 58, 1004 (1936).

BENZALDEHYDE-C^{14}

METHOD I

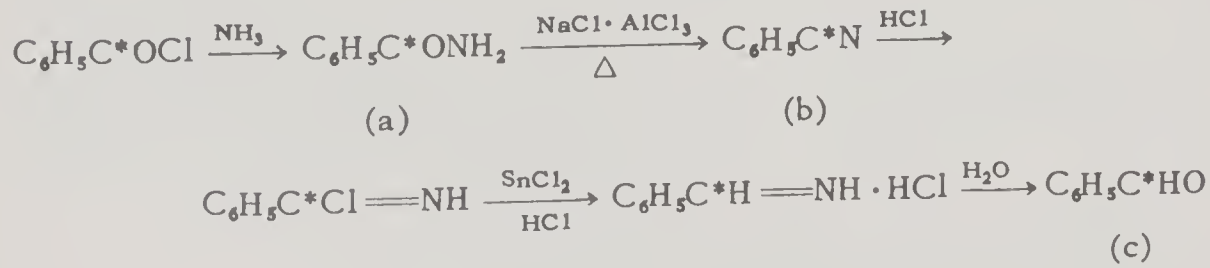


D. G. Ott, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, unpublished work.

A. Procedure (Note 1)

To a stirred solution of 4.5 ml. of anhydrous hydrogen cyanide in 14.2 g. (110 mmoles) of freshly distilled quinoline, maintained at -8° under anhydrous conditions, is added dropwise a solution of 50.0 mmoles of benzoyl-C^{14} chloride in 15 ml. of dry benzene during 10 minutes. The mixture is stirred for 1 hour and stored at room temperature for 24 hours to complete the precipitation of quinoline hydrochloride. The mixture containing 1-benzoyl-C^{14}-1,2-dihydroquinaldonitrile (Note 2) is treated with 150 ml. of 9 *N* sulfuric acid and 15.92 g. of carrier benzaldehyde, and the product is steam-distilled. The distillate (500 ml.) is extracted with five 50-ml. portions of ether, and the extract is washed with four 10-ml. portions of saturated sodium bicarbonate solution (Note 3) and two portions of water, then dried over magnesium sulfate and concentrated in a stream of dry nitrogen. The yield of distilled product is 17.88 g., 52% radiochemical yield based on unrecovered benzoic-C^{14} acid (Note 4).

METHOD II



S. A. Brown and A. C. Neish, Can. J. Biochem. and Physiol., 33, 948 (1955).

A. Procedure (Note 5)

(a) *Benzamide-C^{14}*. Benzoyl-C^{14} chloride is reacted with ammonia in ethanol, and the mixture is evaporated to dryness. The residue is washed with water to remove ammonium chloride (Note 6).

(b) *Benzonitrile-C^{14}*. The benzamide-C^{14} is dehydrated by distillation from sodium aluminum chloride by the method of Norris and Klemka,[1] which follows.

A powdered mixture of equal molecular amounts (200 mmoles) of benzamide and the double salt of aluminum chloride and sodium chloride (Note 7) is introduced into a flask connected to a condenser and receiver. The mixture is heated slowly with a free flame until the evolution of hydrogen chloride nearly ceases (Note 8); then the temperature is raised until no more distillate is collected. The product is redistilled to free it from a small amount of dissolved hydrogen chloride; yield 97%, b.p. 190.7° (cor.).

(c) *Benzaldehyde-C^{14}*. The benzonitrile-C^{14} is converted by the method of Stephen,[2] which follows.

A suspension of 1.5 moles of powdered, anhydrous stannous chloride (Note 9) in absolute ether is saturated with dry hydrogen chloride until two phases form. The nitrile (1 mole) is added with vigorous shaking and, after a few minutes, the crystalline benzylideniimine [-C^{14}] stannichloride (Note 10) separates and is collected on a filter at the end of two hours. The salt is readily hydrolyzed by warm water, and the aldehyde formed is removed by steam distillation or extraction; yield nearly quantitative.

METHOD III

$$C_6H_5C^*OOH \xrightarrow{SOCl_2} C_6H_5C^*OCl \xrightarrow{CH_3OH} C_6H_5C^*OOCH_3 \xrightarrow{NH_2NH_2}$$

$$C_6H_5C^*ONHNH_2 \xrightarrow[C_5H_5N]{C_6H_5SO_2Cl} C_6H_5C^*ONHNHSO_2C_6H_5 \xrightarrow[(CH_2OH)_2]{Na_2CO_3} C_6H_5C^*HO$$

(a) (b) (c)

T. A. Geissman, Atomic Energy Commission Report, UCRL-1233; Nuc. Sci. Abstracts, 5, 4722 (1951).

A. Procedure (Note 11)

(a) *Benzhydrazide-C^{14}*. A solution of 1.6 g. (13.1 mmoles) of benzoic-C^{14} acid in 10 ml. of thionyl chloride is refluxed for 5 hours; then the excess reagent is removed under vacuum. The oily residue is treated with 20 ml. of methanol, and the solution is stored overnight. The solution of methyl benzoate-C^{14} (Note 12) is refluxed for 3 hours with 10 ml.

of 85% hydrazine hydrate; then the solution is concentrated, decolorized with carbon and evaporated to dryness at 50° (15 mm.) (Note 13).

(b) *1-Benzenesulfonyl-2-benzoyl-C^{14}-hydrazine.* To an ice-cooled solution of the benzhydrazide-C^{14} in 10 ml. of dry pyridine is added slowly 3 ml. of benzenesulfonyl chloride. The mixture is stored at 0° for 2 hours, then is treated with cold dilute hydrochloric acid and cooled for 2 hours, and the product is collected, washed and dried. The yield is 3.50 g. (Note 14) after recrystallization from dilute acetic acid (60 ml. acetic acid-125 ml. water).

(c) *Benzaldehyde-C^{14}.* To a solution of the 1-benzenesulfonyl-2-benzoyl-C^{14}-hydrazine in 30 ml. of ethylene glycol at 165° is added 4 g. of anhydrous sodium carbonate. After 1.25 minutes at 165°, the mixture is cooled, diluted, acidified, and extracted with ether. The extract is washed, dried and concentrated; yield 60–65% based on benzoic acid (Note 15).

B. Notes

1. The procedure is an adaptation of that of Reissert[3] and Grosheintz.[4] The method is surveyed at length by Mosettig.[5]

2. The Reissert's compound may be isolated by diluting the mixture with ether and washing with water, dilute acid and bicarbonate solution. The residue obtained by evaporation is crystallized from aqueous alcohol; yield 96%,[4] m.p. 154–155°.

3. Acidification of the alkaline solution yields 0.55 g. of benzoic-C^{14} acid having a molar specific activity essentially equal to that of the starting material.

4. The aldehyde is oxidized quantitatively with silver oxide to benzoic-C^{14} acid for assay. Over-all yields of 98%, as benzaldehyde *p*-nitrophenylhydrazone, have been reported[4] by this method.

5. The only yield data in the isotopic preparations is the statement that the benzaldehyde-C^{14} was converted to cinnamic-β-C^{14} acid in 40% yield based on benzoic acid.

6. Benzamide melts at 130° and boils at 288°; the water solubility is 1 g./74 ml.

7. An intimate mixture of 30 g. of dry sodium chloride and 68 g. of aluminum chloride is heated for 1 hour at 230–250°. The liquid is poured into a beaker and stirred as it solidifies. The double salt is stored in a tightly stoppered bottle and ground before use.

8. The evolution of hydrogen chloride begins at 140–150° and continues at 290–295°, two moles being formed. The reaction is general for both aliphatic and aromatic amides which have no other functional groups sensitive to aluminum chloride.

9. Anhydrous stannous chloride is prepared[6] readily by treating 1 mole of $SnCl_2 \cdot 2H_2O$ with 2 moles of acetic anhydride. The anhydrous salt

separates and is collected, washed with absolute ether and stored indefinitely in a desiccator.

10. The salt, $(C_6H_5C^*H{=}{=}NH \cdot HCl)_2SnCl_4$, may be obtained in the form of white needles from alcohol, and preserved indefinitely, if dry.

11. The procedure is adapted from the method of McFadyen and Stevens[7] for preparing aromatic or heterocyclic aldehydes. The yields are less than those obtained by the Rosenmund method (see below, also *p*-anisaldehyde-C^{14}), but the method is believed to be more reliable.

12. An alternate method of esterification is described by Montgomery; see methyl benzoate-C^{14}.

13. Benzhydrazide melts at 112°.

14. The yield is 97%, assuming no 1,2-bis(benzenesulfonyl)hydrazine is present. No further purification is necessary for the purpose in hand.

15. The oily residue weighs 2.6 g., nearly twice the theoretical amount, but is used satisfactorily for some preparations without further purification (see C_1^{14}-pyribenzamine hydrochloride).

C. Other Preparations

Benzaldehyde-C^{14} has been prepared by Neville[8] by oxidizing benzyl-α-C^{14} alcohol with chromic acid.

Benzaldehyde-C^{14} has been prepared by Bonner[9] and isolated as the 2,4-dinitrophenylhydrazone; yield 95%, m.p. 237–238°. Stilbene-α-C^{14} was ozonized in alcoholic 2,4-dinitrophenylhydrazine sulfate solution, under which conditions the isozonide, formed by rearrangement of the adduct (molozonide), was decomposed by the reagent, forming the hydrazone. The molozonide formation took place with a 4% isotope effect. The molar specific activity agreed with 50% of that of the starting material.

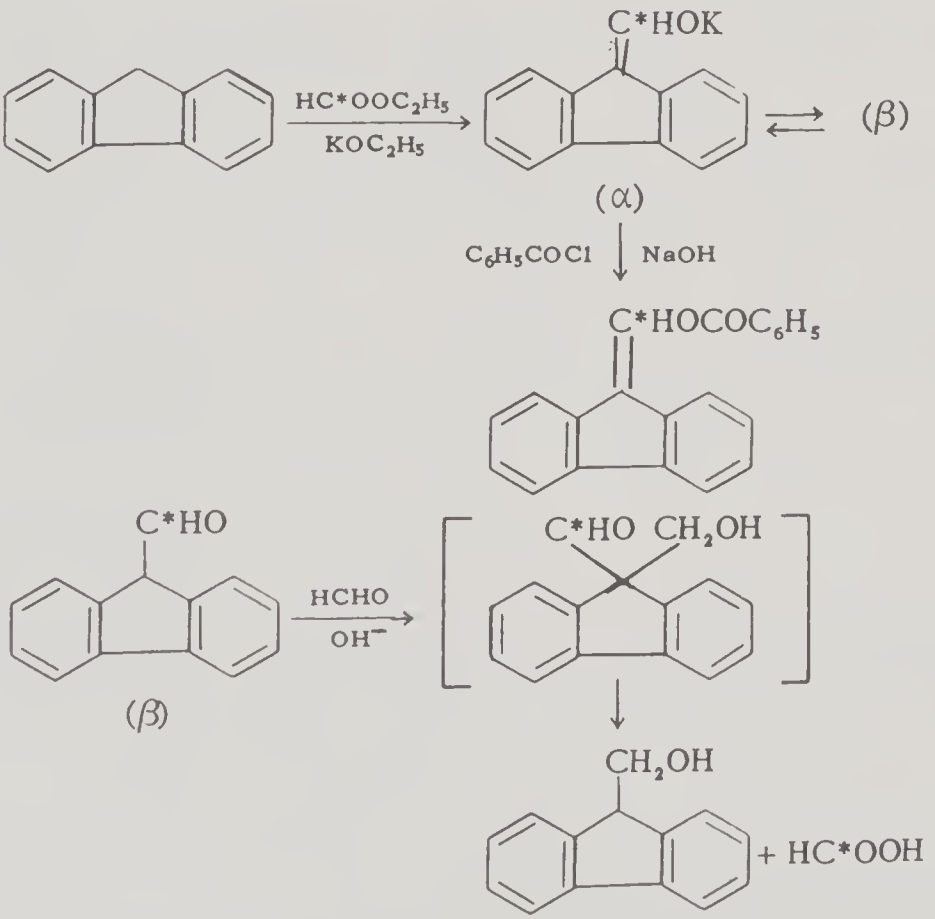
The preparation of benzaldehyde-C^{14} by the Rosenmund reduction[10] of benzoic-C^{14} acid is described by Jerchel;[11] yield of bisulfite derivative 55%.

A new general method for the conversion of nitriles to aldehydes has worked well in some cases where the Stephen reduction[2] and the direct reduction with lithium aluminum hydride have been unsatisfactory.[12] 3-(Methylthio)propionitrile-1-C^{14} was converted[12] to methyl *ortho*-3-(methylthio)propionate-1-C^{14} through the action of methanol and hydrogen chloride, followed by alcoholysis of the iminoester hydrochloride according to the procedure of McElvain;[13] yield 57.6%, b.p. 51–52° (1 mm.). To a boiling 0.33 *M* solution of the *ortho*ester in benzene was added a 0.25 molar equivalent of 1 *M* lithium aluminum hydride in ether, and the mixture was refluxed for 4 hours. The complex was decomposed with 30% Rochelle salt solution, and the benzene extract was dried and distilled to give 3-(methylthio)propionaldehyde-1-C^{14} dimethyl acetal; yield 97%, b.p. 73° (0.9 mm.). 3-(Methylthio)propionaldehyde-1-C^{14} was obtained by hydrolysis with hot dilute hydrochloric acid and ether extraction; the yield at the

usual laboratory scale is 80%, b.p. 60° (12 mm.).[14] The 2,4-dinitrophenylhydrazone melts at 119.5–120.5°.

[1]J. F. Norris and A. J. Klemka, J. Am. Chem. Soc., *62*, 1432 (1940).
[2]H. Stephen, J. Chem. Soc., *127*, 1874 (1925); *Organic Reactions*, Vol. VIII, Wiley, New York, 1954, p. 246; *Organic Syntheses*, Vol. 23, Wiley, New York, 1943, p. 63.
[3]A. Reissert, Ber., *38*, 1603 (1905).
[4]J. M. Grosheintz and H. O. L. Fischer, J. Am. Chem. Soc., *63*, 2021 (1941).
[5]*Organic Reactions*, Vol. VIII, Wiley, New York, 1954, p. 220.
[6]H. Stephen, J. Chem. Soc., *1930*, 2786.
[7]J. S. McFadyen and T. S. Stevens, J. Chem. Soc., *1936*, 584; *Organic Reactions*, Vol. VIII, Wiley, New York, 1954, p. 232.
[8]O .K. Neville, Nucleonics, 7 (4), 45 (1950).
[9]W. A. Bonner and C. J. Collins, J. Am. Chem. Soc., *75*, 3693, 4516 (1953).
[10]*Organic Reactions*, Vol. IV, Wiley, New York, 1948, p. 362.
[11]D. Jerchel, H. Becker and K. Schmeiser, Z. Naturforsch., *8b*, 294 (1953).
[12]C. J. Claus and J. L. Morgenthau, Jr., J. Am. Chem. Soc., *73*, 5005 (1951).
[13]S. M. McElvain and J. W. Nelson, J. Am. Chem. Soc., *64*, 1825 (1942).
[14]G. Barger and F. P. Coyne, Biochem. J., *22*, 1417 (1928).

9-FLUORENECARBOXALDEHYDE-C^{14}



J. G. Burr, Jr., J. Am. Chem. Soc., 73, 823 (1951).

A. Procedure (Note 1)

To a stirred mixture, under nitrogen, of absolute ether and the potassium ethoxide prepared from 0.5 g. (12.8 mmoles) of potassium is added an ethereal solution of 2.0 g. (12.0 mmoles) of purified fluorene and 1.0 g. (13.5 mmoles) of ethyl formate-C^{14}. Under gentle heating the solid quickly dissolves (Note 2). In about 1 hour the liquid is hydrolyzed with 18 ml. of cold water, and 0.54 g. (27%) of fluorene is recovered. The alkaline solution is freed of ether, cooled and acidified with dilute sulfuric acid to precipitate the product (Note 3), which may be recrystallized from petroleum ether or carbon tetrachloride. The compound is assayed as 9-fluorenylidenemethyl benzoate m.p. 157–158° (Note 4).

The aldehyde has been used in a study of the mechanism of the reaction in which the action of excess aqueous basic formaldehyde forms 9-fluorenemethanol (Note 5).

B. Notes

1. The procedure is that of Wislicenus and Waldmuller.[1] The yield is 80–90% with potassium ethoxide as the condensing agent, or 65% with the sodium salt. The compound does not keep well and may be used as the aqueous solution resulting from hydrolysis of the reaction mixture.

2. In the absence of a nitrogen atmosphere, Wislicenus observed that fluorenone forms upon standing, probably through reaction with atmospheric oxygen.

3. The precipitate under these conditions is the β-formylfluorene (sinters at 70°), which may be completely converted to the α-formylfluorene at 90° (oil, b.p. 193–193.5° at 12 mm.).

4. A sodium hydroxide solution of 9-fluorenecarboxaldehyde-C^{14} is shaken with benzoyl chloride, and the separated solid is recrystallized from dilute alcohol. This derivative is useful for the assay of ethyl formate-C^{14}.

5. The yield of purified 9-fluorenemethanol, which has not been an easily available compound by any other method,[2] is over 67% by this procedure of Brown and Bluestein.[3] To a vigorously stirred alkaline solution of 9-fluorenecarboxaldehyde-C^{14} was added slowly an excess of 10% formalin solution. The precipitated oil crystallized and was collected in nearly quantitative yield. The nonisotopic 9-fluorenemethanol melted at 99–100° after two recrystallizations from hexane. The aqueous filtrate was diluted with carrier sodium formate, concentrated, acidified with hydrochloric acid and treated with an excess of *S*-benzylthiuronium chloride solution to precipitate *S*-benzylthiuronium formate-C^{14}, m.p. 148–149°. The excess formaldehyde, assayed as the dimedon derivative, was

nonisotopic, thus ruling out any exchange reactions. The reaction has the appearance of a crossed Cannizzaro reaction,[4] but is apparently an aldol condensation followed by alkaline cleavage of the unstable tertiary aldehyde with displacement of the formyl group (see benzoylphenylacetaldehyde-1-C^{14}). Other aldehydes do not react sufficiently fast in competition with the decomposition of 9-fluorenecarboxaldehyde in basic solution[3] to form a product. A postulated intermediate was actually isolated in the case of phenyl 9-fluorenyl ketone; see Other Preparations.

9-Fluorenemethanol is dehydrated by strong alcoholic alkali to 9-methylenefluorene, isolated as the polymer; and 9-fluorenecarboxaldehyde in formalin solutions of higher alkaline concentration also gives 9-methylenefluorene instead of 9-fluorenemethanol.[5]

C. Other Preparations

The reaction of alkaline formaldehyde-C^{14} with a similar carbonyl compound containing a substituent phenyl group rather than hydrogen has been described by Burr.[5] A mixture of 0.50 g. (1.67 mmoles) of 9-hydroxymethyl-9-fluorenyl phenyl ketone (prepared from alkaline formaldehyde solution and phenyl 9-fluorenyl ketone), 5 ml. of alcohol, 7 ml. (23 mmoles) of 10% formaldehyde-C^{14} solution and 5 ml. of 50% aqueous potassium hydroxide is stirred for 30 minutes. As the product, presumably 9-methylene-C^{14}-fluorene, polymerizes with extreme readiness, the precipitate (0.25 g.) is collected, dissolved in hexane and brominated, giving 0.15 g. of 9-bromo-9-(bromomethyl-C^{14})fluorene, m.p. 143°.[6] The molar specific activities of the product and recovered formaldehyde are 94.7% and 93.5% of that of the starting material, respectively, indicative of an equilibrium between the aldehyde, ketone and hydroxyketone.

[1]W. Wislicenus and M. Waldmuller, Ber., *42*, 785 (1909).
[2]W. G. Brown and B. Bluestein, J. Am. Chem. Soc., *62*, 3256 (1940).
[3]W. G. Brown and B. A. Bluestein, *ibid.*, *65*, 1082 (1943).
[4]*Organic Reactions*, Vol. II, Wiley, New York, 1944, p. 100.
[5]J. G. Burr, Jr., J. Am. Chem. Soc., *74*, 1717 (1952).
[6]H. Wieland, F. Reindel and J. Ferrer, Ber., *55*, 3317 (1922).

2- PROPANONE-1-C^{14}

$$CH_3CHO \xrightarrow{C^*H_3MgI} \underset{(a)}{C^*H_3CHOHCH_3} \xrightarrow[H_2CrO_4]{[o]} \underset{(b)}{C^*H_3COCH_3}$$

A. Roe and E. L. Abenesius, J. Am. Chem. Soc., *74*, 2402 (1952).

A. Procedure

(a) *2-Propanol-1-C^{14}*. The methyl-C^{14} iodide, prepared from 20 mmoles of methanol-C^{14} (Note 1) and dried over phosphorus pentoxide-ether mixture, is distilled on a vacuum line into a flask containing 20 mmoles of magnesium and a magnetic stirrer (Note 2). The flask is removed from the vacuum line and fitted with a reflux condenser and dropping funnel. To the stirred Grignard solution is slowly added a solution of 25 mmoles of acetaldehyde in 5 ml. of ether. After being stirred for 1 hour, the mixture is evaporated to dryness under reduced pressure, and the residue is hydrolyzed with 10 ml. of water, which is added by distillation. The mixture of water and product is distilled into a 100-ml. volumetric flask.

(b) *2-Propanone-1-C^{14}*. To the aqueous solution of 2-propanol-1-C^{14} is added a mixture of 20 ml. of 2 *M* chromic acid, 10 ml. of 2 *N* perchloric acid and sufficient water to make the volume 100 ml. The solution is heated for 48 hours in a constant temperature bath at 40°. The yield, determined by precipitating the 2,4-dinitrophenylhydrazone from an aliquot, is 60–75% based on methanol. The product is isolated from the oxidizing mixture by vacuum distillation, followed by redistillation from basic solution. The molar specific activity is the same as that of the starting methanol-C^{14} (Note 3).

B. Notes

1. Preparation was by the method of Tolbert (see methanol-C^{14}) which usually gives yields of 95%.

2. The ether required for preparing the Grignard reagent is distilled with the halide.

3. The yield from 0.8 ml. of methanol-C^{14} was 0.66 ml. of 2-propanone-1-C^{14}.

Degradation with sodium hypoiodite produced iodoform-C^{14} (85% yield), exhibiting a 6% greater molar specific activity that that of the accompanying acetate-2-C^{14}. The apparent greater stability of the C^{12}-C^{12} bond in this case is contrary to previous observations in isotope effect studies.[1] A portion of the acetate was assayed as the *S*-benzylthiuronium salt,[2] recrystallized from ethyl acetate; another portion was converted to the lithium salt and pyrolyzed to acetone, which was assayed as the 2,4-dinitrophenylhydrazone or converted to iodoform.

A repetition of this work by a number of investigators,[3] employing four different procedures and 2-propanone-1-C^{14} prepared by two methods, indicated that there was no measurable intramolecular or intermolecular isotope effect. The ketone was assayed as the 2,4-dinitrophenyl hydrazone or the semicarbazone, and the acetic acid as the *S*-(1-naphthyl-

methyl)thiuronium salt or the *p*-nitrobenzyl ester. The Schmidt reaction[4] with 2-propanone-1-C^{14} gave a small isotope effect in the usual direction, contrary to the findings of Roe.[1] The precipitation of acetone as the Deniges complex and subsequent degradation to iodoform and acetate have been described by Lewis[5] and Lerner.[6]

[1]A. Roe, Chem. Eng. News, *31*, 3980 (1953).
[2]J. J. Donleavy, J. Am. Chem. Soc., *58*, 1004 (1936).
[3]G. A. Ropp, W. A. Bonner, M. T. Clark and V. F. Raaen, J. Am. Chem. Soc., *76*, 1710 (1954).
[4]*Organic Reactions*, Vol. III, Wiley, New York, 1946, p. 327.
[5]K. Lewis and S. Weinhouse, J. Am. Chem. Soc., *73*, 2500 (1951).
[6]A. B. Lerner, J. Biol. Chem., *181*, 281 (1949); D. D. Van Slyke, J. Biol. Chem., *32*, 455 (1917).

2-PROPANONE-2-C^{14}

$$CH_3C^*OOLi \xrightarrow{\Delta} CH_3C^*OCH_3 + Li_2C^*O_3$$

A. Roe and J. B. Finlay, J. Am. Chem. Soc., *74*, 2442 (1952).

A. Procedure (Note 1)

Dry lithium acetate-1-C^{14} (2.5 mmoles), contained in a platinum boat within a platinum sleeve, is heated in a Pyrex combustion tube at 490° (electric furnace) for about 20 minutes. The product is swept out in a stream of nitrogen into a cold trap (Dry Ice or liquid air), or into a saturated solution of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid. The yield is 80% or better (Note 2), and the molar specific activity is unchanged from that of the acetate (Note 3).

B. Notes

1. The procedure is an adaptation of the method of Kronig.[1]

2. Zabin[2] has reported 80–95% yields of 2-propanone-1,3-C_2^{14} from lithium acetate-2-C^{14}.

3. The method has been used by Bloch.[3] There is no isotope effect in this pyrolytic reaction. The molar specific activity of the lithium carbonate-C^{14} formed is about 2% less than that of the starting acetate-C^{14}, due to slight dilution by carbon dioxide from methyl carbons resulting from decomposition accompanying the pyrolysis.[4] A gravimetric determination of acetone by means of the Deniges complex has been described by Van Slyke; see 2-propanone-1-C^{14}, Note 3.

C. Other Preparations

2-Propanone-2-C^{14} has been prepared by Dauben[5] and Grosse[6] (yield 0.432 g., 95%) and Logan[7] (2-mmole scale) by pyrolysis of barium acetate-1-C^{14} at 500° in vacuum, while Coon[8,9] raised the temperature to 530° (yield 74%). Coon and Gurin[9] have pyrolyzed calcium acetate-1-C^{14} at 430–490° in a stream of dry heated nitrogen in a 2 × 15-cm. tube, collecting the product in a trap cooled with liquid nitrogen.

2-Propanone-1,3-C_2^{14} is prepared[2,3] in a similar manner by pyrolysis of lithium acetate-2-C^{14}; yield 80–95%.

2-Propanone-1,3-C_2^{14} has been prepared in a similar manner by pyrolysis of barium acetate-2-C^{14} at 500° in vacuum. Price[10] has trapped the ketone as the 2,4-dinitrophenylhydrazone, m.p. 124.5–125.0° (yield 80%), then, following assay, decomposed the derivative with dilute sulfuric acid and steam distillation, forming an aqueous solution of the product. Sakami[11] has purified the product of a similar preparation by distillation over acid permanganate solution, conversion to the *p*-nitrophenylsemicarbazone and recovery by distillation from 2 *N* sulfuric acid. The purity was determined by the salicylaldehyde method[12] (100 ± 2%). Koshland[13] has reported that the specific activity of the product obtained by pyrolysis at 530° in vacuum was only 77% of the expected value, and some activity was found in the barium carbonate. Aronoff[14] has reported that conducting the pyrolysis at 450° in a stream of argon leads to the formation of a purified barium carbonate-C^{14} having more than 8% of the initial activity. This value, however, dropped to 0.86% when the heating was at 530° for 10 minutes. The rate of acetone formation by distillation of calcium acetate has been studied thoroughly by Ardagh,[15] who found that yields suffer unless the product is removed as soon as formed. Directions and a diagram of a suitable apparatus are given by Calvin[16] for preparing isotopic acetone (yield 70%) by pyrolysis at 525° for 10 minutes in a stream of inert gas. The residual barium carbonate, under these conditions for degrading acetic acid, contained 3.5% of the original C-2 (15% at 450°). The isotopic preparation[16] and purification by the method of Sakami[11] have been reported by Siegel[17] and Rudney.[18]

Bell[19] has reported that the pyrolysis of a mixture of barium acetate-1-C^{13} and barium formate formed nonisotopic acetaldehyde and 2-propanone-2-C^{13}.

The preparation of barium acetate-1-C^{14} has been described by Dauben.[5] An aqueous solution containing 12.4 mmoles of sodium acetate-1-C^{14} and 17.9 mmoles of *p*-toluenesulfonic acid in 50 ml. of water was distilled until crystals formed in the residue. Following the addition and distillation of two 25 ml. portions of water, the combined distillate was titrated

with 0.18 *N* barium hydroxide (phenolphthalein) and evaporated; yield 99.2%.

[1]W. Kronig, Angew. Chem., *37*, 667 (1924).
[2]I. Zabin and K. Bloch, J. Biol. Chem., *185*, 117 (1950).
[3]K. Bloch, L. C. Clark and I. Harary, J. Biol. Chem., *211*, 687 (1954).
[4]A. Roe and E. L. Albenesius, J. Am. Chem. Soc., *74*, 2402 (1952).
[5]W. G. Dauben and C. W. Vaughan, Jr., J. Am. Chem. Soc., *75*, 4651 (1953).
[6]A. V. Grosse and S. Weinhouse, Science, *104*, 2704 (1946).
[7]A. V. Logan and J. Murray, J. Am. Chem. Soc., *74*, 2436 (1952).
[8]M. J. Coon, J. Biol. Chem., *187*, 71 (1950).
[9]M. J. Coon and S. Gurin, J. Biol. Chem., *180*, 1159 (1949).
[10]T. D. Price and D. Rittenberg, J. Biol. Chem., *185*, 449 (1950).
[11]W. Sakami, J. Biol. Chem., *187*, 369 (1950).
[12]O. Eichler and H. Hindemith, Biochem. Z., *314*, 73 (1943).
[13]D. Koshland, Jr., and F. H. Westheimer, J. Am. Chem. Soc., *71*, 1139 (1949).
[14]S. Aronoff, V. A. Haas and B. Fries, Science, *110*, 476 (1949).
[15]E. G. R. Ardagh, A. D. Barbour, G. E. McClellan and E. W. McBride, Ind. Eng. Chem., *16*, 1133 (1924).
[16]M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 248.
[17]J. M. Siegel, J. Biol. Chem., *208*, 205 (1954).
[18]H. Rudney, J. Biol. Chem., *210*, 361 (1954).
[19]J. Bell and R. I. Reed, J. Chem. Soc., *1952*, 1383; Nature, *165*, 402 (1950).

1,3-DIHYDROXY-2-PROPANONE-2-C^{14}

METHOD I

$$HOCH_2C^*OOH \xrightarrow{AcCl} \underset{(a)}{CH_3COOCH_2C^*OOH} \xrightarrow{SOCl_2} \underset{(b)}{CH_3COOCH_2C^*OCl}$$

$$\xrightarrow{CH_2N_2} \underset{(c)}{CH_3COOCH_2C^*OCHN_2} \xrightarrow{H_2SO_4} \underset{(d)}{(HOCH_2)_2C^*O}$$

S. Gatenbeck, Acta Chem. Scand., *9*, 709 (1955).

A. Procedure

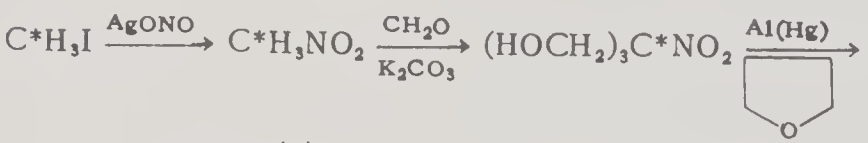
(a) *Acetoxyacetic-1-C^{14} Acid, (Glycolic-1-C^{14} Acid Acetate)*. A mixture of 5.0 g. (66 mmoles) of glycolic-1-C^{14} acid and 10 g. (127 mmoles) of acetyl chloride is refluxed for 45 minutes, then evaporated on a water-bath at reduced pressure. The residual product melts at 66–68°.

(b) *Acetoxyacetyl-1-C^{14} Chloride*. A mixture of the acetoxyacetic-1-C^{14} acid and 11.2 g. (102 mmoles) of thionyl chloride is refluxed for 1.5 hours, then concentrated under reduced pressure. The product is fractionally distilled; yield 8.0 g. (89%), b.p. 52–54° (12–14 mm.).

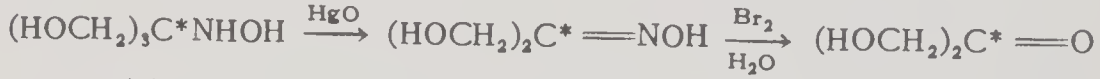
(c) *1-Acetoxy-3-diazo-2-propanone-2-C*^{14}. To a stirred, ice-cold solution of 100 mmoles of diazomethane in 150 ml. of absolute ether is added dropwise a solution of 3.50 g. (25.6 mmoles) of acetoxyacetyl-1-C^{14} chloride in 5 ml. of absolute ether during 1 hour. The solution is stirred at room temperature for 1 hour, then concentrated at a water-bath under reduced pressure.

(d) *1,3-Dihydroxy-2-propanone-2-C*^{14}. The crude 1-acetoxy-3-diazo-2-propanone-2-C^{14} is dissolved in 10 ml. of absolute alcohol. To a 3.0-ml. aliquot, diluted with an equal volume of water, is added dropwise 3 ml. of 8 *N* sulfuric acid, the mixture being cooled when the nitrogen evolution becomes violent. The solution is stored at room temperature until the gas evolution is essentially over, then heated at 75° for 45 minutes. The cooled mixture is diluted with water and neutralized with powdered barium carbonate. The precipitate is separated by centrifuging, and the filtered solution is evaporated at 35° under reduced pressure. The residue is stored for 2 days in a vacuum desiccator over sulfuric acid, then triturated with acetone; yield 0.42 g. (53% based on glycolic acid), m.p. 75–76°.

METHOD II



(a) (b)



(c) (d) (e)

H. R. V. Arnstein and R. Bentley, J. Chem. Soc., *1951*, 2385.

A. Procedure (Note 1)

(a) *Nitromethane-C*^{14} (Note 2). Methyl-C^{14} iodide (6.1 g.) is vacuum-distilled into a flask, containing 12.0 g. of silver nitrite and 13.4 g. of carrier nitromethane, and which is equipped with a Dry Ice-carbon tetrachloride-cooled reflux condenser and a gas inlet. After an initial exothermic reaction, the mixture is heated at 85–90° for 1.25 hours. The Dry Ice mixture is replaced by an ice-salt mixture, and the by-product, methyl-C^{14} nitrite, is removed in a stream of dry air into a liquid nitrogen-cooled trap (Note 3). The product is then vacuum-distilled into another trap cooled with liquid nitrogen; yield 14.64 g., 73.6% radiochemical yield (Note 4).

(b) *2-(Hydroxymethyl)-2-nitro-1,3-propanediol-2-C*^{14}. Nitromethane-C^{14} (4.67 g.) is mixed with 17.50 g. of 40% formaldehyde solution and 0.050 g. of anhydrous potassium carbonate. When the temperature rises to 50°, the flask is cooled to control the reaction. After 5 minutes, 50 ml. of water is added, and the solution is passed in succession through columns of Amberlite IR-100 and Deacidite E (Note 5). The columns are eluted with 2 l. of water, and the solution is evaporated under vacuum below 60°. The residue is dissolved in 100 ml. of warm ethyl acetate and filtered, and the solution is evaporated under reduced pressure. The white crystalline residue is washed out with chloroform to yield 8.35 g. of product, m.p. 162–165°. A second crop (1.25 g.) is obtained by concentrating the chloroform washings, making the total yield 83.5% (Note 6).

(c) *2-(Hydroxyamino)-2-(hydroxymethyl)-1,3-propanediol-2-C*^{14}, (N-[*Tris-(hydroxymethyl)methyl-C*^{14}]*hydroxylamine*). A solution of 2.9369 g. of the nitro alcohol and 25 ml. of tetrahydrofuran is added to aluminum amalgam, prepared from 12 g. of aluminum foil, under 75 ml. of absolute ether (Note 7). Water (2 ml.) is added, and the stirred mixture is refluxed for 15 minutes; then another 2-ml. portion of water is added, and the mixture is stirred again for 45 minutes. The precipitate is separated by centrifuging and washed with fifteen 40-ml. portions of water; the combined solution is evaporated under reduced pressure below 5.5°. The crystalline residue is washed out with absolute ether containing a little ethanol; yield 1.675 g. of white crystalline powder (63.5%), m.p. 136–142° (Note 8).

(d) *1,3-Dihydroxy-2-propanone-2-C*^{14} *Oxime*. To a stirred suspension of 1.0 g. of the hydroxylamine in 20 ml. of methanol at 65° is added in portions (until a pronounced yellow color persists) a paste of yellow mercuric oxide, freshly prepared from 10 g. of mercuric chloride and suspended in methanol. The mixture is stirred for 2 hours at 60–65°, then filtered, and the residue is washed with methanol. The combined solution is evaporated at 40° under reduced pressure to yield an oil which slowly crystallizes (Note 9).

(e) *1,3-Dihydroxy-2-propanone-2-C*^{14}. To a stirred mixture of the crude 1,3-dihydroxy-2-propanone-2-C^{14} oxime and 8.0 ml. of water is added an excess of bromine (0.5 ml.) dropwise over a period of 10 minutes. The mixture is stirred for 1.5 hours; then the colorless solution is treated with an excess of lead carbonate, and the insoluble salts are filtered off and washed. The combined solution is passed in succession through columns of Amberlite IR-100 and Deacidite E (Note 10) by washing with 2 l. of water. The eluate is evaporated under reduced pressure below 40° and the residual yellow oil is dissolved in 5 ml. of ethanol and treated with 50 ml. of ether. The supernatant liquid is decanted from the precipitated oil through a filter and evaporated to dryness. The process is repeated with 3 ml. of ethanol, 30 ml. of ether and a little charcoal.

The residue, which crystallizes readily on seeding, is dried overnight in a vacuum desiccator containing phosphorus pentoxide, then is mixed with 3 ml. of 1-butanol and filtered. The residue is washed with a little butanol-ether (1 : 2); yield 0.105 g., m.p. 76–78° (Note 11). The mother liquor deposits further product upon standing, making the total chemical yield 27.2% from (c) and 14.2% from (a). The mother liquor is reworked with 1.0 g. of carrier to give 0.900 g. of diluted product, making the radiochemical yield 41.1% from (c) and 21.8% from (a).

METHOD III

$$C^*H_3NO_2 \xrightarrow[K_2CO_3]{CH_2O} (HOCH_2)_3C^*NO_2 \xrightarrow{NaOCH_3} [(HOCH_2)_2C^*NO_2]^-Na^+ \xrightarrow{H_2SO_4}$$

(a)

$$(HOCH_2)_2C^*{=}{=}O$$

(b)

H. R. V. Arnstein and R. Bentley, J. Chem. Soc., *1951*, 2385.

A. Procedure

(a) *Sodium 2-Nitro-1,3-propanediol-2-C*^{14}. Nitromethane-C^{14} (1.718 g.) is mixed with 6.450 g. of 40% formaldehyde solution and 0.050 g. of potassium carbonate, and when the reaction temperature reaches 50°, the flask is cooled with water (see Method II). When the reaction is over, the solvent is evaporated under vacuum below 50°. The residue is dissolved in 50 ml. of methanol and added to a solution of 1 g. of sodium in 50 ml. of methanol. After cooling for 1 hour at 0°, the white precipitate is collected; yield 4.773 g., 91% (Note 12).

(b) *1,3-Dihydroxy-2-propanone-2-C*^{14} (Note 13). A solution of the sodium 2-nitro-1,3-propanediol-2-C^{14} in 35 ml. of water is added, over a period of 15 minutes, to a stirred ice-cooled solution of 3 ml. of concentrated sulfuric acid and 5 ml. of water (Note 14). The mixture is stirred for 10 minutes at room temperature, then slowly added to a stirred suspension of 10 g. of calcium carbonate in 50 ml. of water. The precipitate is filtered off and washed with water. The combined solution is mixed with 1.99 g. of carrier 1,3-dihydroxy-2-propanone and purified by chromatographic absorption as described in Method II; yield 1.025 g., m.p. 85–89° (Note 15). The radiochemical yield is 30% (10.1% based on nitromethane-C^{14}).

B. Notes

1. The procedure is an improvement of the method of Piloty.[1] The 1,3-labeled compounds are available through formaldehyde-C^{14}.

2. The method is that of Sowden,[2] who achieved 71% yield of nitromethane-C^{14} and 16% yield of methyl-C^{14} nitrite. The procedure has been used by Swan.[5]

3. This by-product is sealed into a tube fitted with a break-seal for convenient storage.

4. The product is assayed as β-nitrostyrene-β-C^{14}.[3] To a stirred, ice-cooled mixture of 0.6 g. of nitromethane-C^{14}, 1.06 g. of redistilled benzaldehyde and 2 ml. of methanol is added slowly 0.42 g. of sodium hydroxide in 1.5 ml. of ice-water. Methanol (3 ml.) is added, followed a few minutes later by 8 ml. of water. The solution is added to a mixture of 2 ml. of concentrated hydrochloric acid and 3 ml. of water. The yellow oil is separated, washed with water and crystallized by the addition of alcohol. The pale yellow crystals melt at 56°, after recrystallization from alcohol. Also see β-nitrostyrene-α-C^{14}.

5. Both columns are 4.5 $cm.^2 \times 40$ cm., and the Amberlite is used in the acid form. Purification may also be accomplished by placing a methanol solution of the crude compound on a column of alumina and fractionally eluting with methanol.

6. Recrystallization from nitromethane gives long white needles, m.p. 176°. The melting points variously given in the literature (144° to 162°) are probably due to inclusion of traces of alkaline catalyst which causes reversible decomposition.[4]

7. Strips of aluminum foil are warmed with 10% sodium hydroxide solution until a vigorous evolution of hydrogen takes place for several minutes. The foil is washed with water and alcohol. Sufficient 2% mercuric chloride solution is added to completely cover the aluminum and, after 2 minutes, the amalgam is washed successively with water, alcohol and ether. The amalgam is covered with ether and is ready for immediate use.

8. The reduction with aluminum amalgam appears to be more reliable than with sodium amalgam.[1,6] With aqueous solution the yield is up to 20%. Hydrogenation over palladium-barium sulfate in methanol is unsatisfactory.

9. Purification is unnecessary for the next step. The oily contaminant is not removed by solvents, but purification may be effected through the insoluble copper compound, 65% yield.

10. The columns are 2 $cm.^2 \times 25$ cm. and 30 cm., respectively. The Amberlite is used in the hydrogen cycle.

11. Microblock, rapid heating.

12. The product is precipitated in nearly quantitative yield, if the nitromethane-C^{14} is condensed with paraformaldehyde in methanol and then added to sodium methoxide in methanol (see 1,2,3-propanetriol-1,-3-C_2^{14}, Method III).

Directions are given by Arnstein for converting the salt to 2-nitro-1,3-propanediol by reaction with salicylic acid in absolute ether. The 2-amino-1,3-propanediol oxalate, formed by hydrogenation over palladium-barium sulfate in the presence of oxalic acid dihydrate as a trapping agent, did not lead to the isolation of glycerol (tribenzoate) when treated with barium chloride and nitrous acid.

13. This method provides a rapid synthesis, when dilution of radioactivity may be tolerated.

14. A blue-green color develops, and there is gas evolution with each addition.

15. The product is characterized by the melting point (slow heating in an air-bath) and molar specific activity of three derivatives: phenylosazone, 2,4-dinitrophenylhydrazone and methylglyoxal disemicarbazone.

[1]O. Piloty, *Ber.*, *30*, 3161 (1897)
[2]J. C. Sowden, J. Biol. Chem., *180*, 55 (1949); Science, *109*, 229 (1949).
[3]G. A. Ropp, V. F. Raaen and A. J. Weinberger, J. Am. Chem. Soc., *75*, 3694 (1953).
[4]I. M. Gorski and S. P. Makarow, Ber., *67B*, 996 (1934).
[5]G. A. Swan and D. Wright, J. Chem. Soc., *1954*, 381.
[6]J. Cason and F. S. Prout, J. Am. Chem. Soc., *71*, 1218 (1949).

1,3-DIPHENYLPROPANETRIONE-1-C^{14}

$$C_6H_5C^*OCH{=}CHC_6H_5 \xrightarrow{Br_2} C_6H_5C^*OCHBrCHBrC_6H_5 \xrightarrow{NaOCH_3}$$

$$C_6H_5C^*OCH{=}\underset{\displaystyle OCH_3}{\underset{|}{C}}C_6H_5 \xrightarrow{HCl} C_6H_5C^*OCH{=}COHC_6H_5 \rightleftharpoons$$

$$C_6H_5C^*OCH_2COC_6H_5 \xrightarrow[Cat.]{\overset{CH_2CO}{\underset{CH_2CO}{\diagup\!\diagdown}}NBr} C_6H_5C^*OCHBrCOC_6H_5 \xrightarrow{NaOAc}$$

(a)

$$C_6H_5C^*O\underset{\displaystyle OCOCH_3}{\underset{|}{C}}HCOC_6H_5 \xrightarrow{Br_2} C_6H_5C^*O\underset{\displaystyle OCOCH_3}{\underset{|}{C}}BrCOC_6H_5 \xrightarrow[\Delta]{} C_6H_5C^*OCOCOC_6H_5$$

(b) (c)

J. D. Roberts, D. R. Smith and C. E. Lee, J. Am. Chem. Soc., 73, 618 (1951).

A. Procedure

(a) *1,3-Diphenyl-1,3-propanedione-1-C^{14}*. The yield of product from 35 g. of acetophenone-1-C^{14} is 40.5 g. (62%), as prepared by the procedures of Kohler and Chadwell (see 2-benzylideneacetophenone-1-C^{14}) and of Allen, Abell and Normington.[1]

(b) *2-Acetoxy-1,3-diphenyl-1,3-propanedione-1-C^{14}*. A mixture of 40.5 g. (0.18 mole) of 1,3-diphenyl-1,3-propanedione-1-C^{14}, 72 g. (0.4 mole) of *N*-bromosuccinimide, a few mg. of benzoyl peroxide and 300 ml. of carbon tetrachloride is refluxed for 20 hours, while being irradiated by an ultraviolet lamp. The solid is filtered off and washed with hot carbon tetrachloride. The combined filtrate is washed with water, dried over magnesium sulfate and evaporated. The residue (Note 2), 2-bromo-1,3-diphenyl-1,3-propanedione-1-C^{14}, is heated for 1.5 hours with 33 g. of fused sodium acetate in 130 ml. of glacial acetic acid. The cooled mixture is diluted with water, and the product is collected, dried, distilled at 5 μ and crystallized from ligroin; yield 26 g. (51%), m.p. 86–89°.

(c) *1,3-Diphenylpropanetrione-1-C^{14}*. To a stirred, cooled (4–7°) solution of 7 g. (25 mmoles) of 2-acetoxy-1,3-diphenyl-1,3-propanedione-1-C^{14} in 50 ml. of chloroform is added a solution of 1.5 ml. of bromine in 15 ml. of chloroform over a 30-minute period. Hydrogen bromide is removed by bubbling dry air through the mixture and, after several hours at room temperature, the solvent is distilled under reduced pressure. The residue is heated for about 15 minutes under reduced pressure with a soft flame, while acetyl bromide is evolved. The residue is distilled, and the product is recrystallized from anhydrous ligroin; yield 5.0 g. (84%), m.p. 68–69° (Note 4). The hydrate (m.p. 88–89°) is formed by recrystallizing the product from diluted acetic acid.[2]

B. Notes

1. The method of Wieland and Bloch[3] for the preparation of 1,3-diphenylpropanetrione from 1,3-diphenyl-1,3-propanedione (using nitrogen tetroxide) has been reported to be unsatisfactory by two groups of investigators,[4,5] and the method of Kohler and Erickson[4] is believed to be perferable to that of Bigelow and Hanslick.[6]

2. Kohler and Erickson[4] have reported obtaining the bromide in 78% yield by halogenating with bromine.

3. Kohler and Erickson[4] have reported a yield of 79%, m.p. 94°, after recrystallization from methanol.

4. The product is hygroscopic.[6]

C. Other Preparations

Starting with 4′-methoxychalcone-C^{14}, (2-benzylidene-4′-methoxyacetophenone-1-C^{14}), a similar series of compounds is prepared with the following yields and melting points:

2,3-Dibromo-4′-methoxy-3-phenylpropiophenone-1-C^{14}, 73%, m.p. 161–162°.

1-(*p*-Methoxyphenyl)-3-phenyl-1,3-propanedione-1-C^{14}, 95.7%, m.p. 131–132°.

2-Bromo-1-(*p*-methoxyphenyl)-3-phenyl-1,3-propanedione-1-C^{14}, 80.5%, m.p. 127–128°. This bromination is carried out in the more conventional manner.[7] To a solution of 17.5 g. (69 mmoles) of 1-(*p*-methoxyphenyl)-3-phenyl-1,3-propanedione-1-C^{14} in 60 ml. of chloroform at 4–7° is added a solution of 4.0 ml. of bromine in 40 ml. of chloroform over a period of 2 hours. The hydrogen bromide is removed with a stream of dry air; after 2 hours at room temperature, the solvent is removed under reduced pressure and the product is recrystallized from benzene-hexane.

2-Acetoxy-1-(*p*-methoxyphenyl)-3-phenyl-1,3-propanediol-1-C^{14}. The bromide is refluxed for 20 minutes with 8.0 g. of freshly fused potassium acetate in 70 ml. of acetic acid.

2-Acetoxy-2-bromo-1-(*p*-methoxyphenyl)-3-phenyl-1,3-propanedione-1-C^{14}. A solution of the residual crude acetoxy compound in 80 ml. of chloroform is treated, over a period of 2 hours at 5–10°, with a solution of 2.9 ml. of bromine in 40 ml. of chloroform. After standing overnight, the solvent is removed under reduced pressure.

1-(*p*-Methoxyphenyl)-3-phenylpropanetrione-1-C^{14}, 71%, m.p. 66–66.5°. The residue of crude bromo-acetoxy compound is heated at 100° (30 mm.) until the evolution of acetyl bromide ceases (30 minutes). The product is distilled at 5 μ and crystallized from ether; yield 10.5 g.

[1]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 205.
[2]R. de Neufville and H. V. Pechmann, Ber., *23*, 3375 (1890).
[3]H. Wieland and S. Bloch, Ber., *37*, 1524 (1904).
[4]E. P. Kohler and J. L. E. Erickson, J. Am. Chem. Soc., *53*, 2301 (1931).
[5]J. D. Roberts, D. R. Smith and C. E. Lee, *ibid.*, *73*, 618 (1951).
[6]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 244.
[7]F. J. Pond and A. S. Shoffstall, J. Am. Chem. Soc., *22*, 658 (1900).

3-HYDROXY-2-BUTANONE-1-C^{14}
(C_1^{14}-Acetoin)

$$CH_3CHOHCN \xrightarrow{C^*H_3MgI} CH_3CHOHCOC^*H_3$$

R. O. Brady, J. Rabinowitz, J. Van Baalen and S. Gurin, J. Biol. Chem., *193*, 137 (1951).

Procedure

To 2.13 g. (30 mmoles) of lactonitrile, in a three-necked flask fitted with an efficient stirrer, reflux condenser and dropping funnel, is added slowly 33 mmoles of nonisotopic methylmagnesium iodide in 25 ml. of absolute ether. The mixture is stirred vigorously until the evolution of methane ceases; then a similar amount of methylmagnesium-C^{14} iodide in ether is added slowly. The mixture, containing the insoluble double magnesium salt, is stirred with gentle warming for 1 hour, then cooled and decomposed with a minimal volume of saturated sodium chloride solution. The mixture is adjusted to pH 2 with concentrated hydrochloric acid, and the aqueous layer is separated and continuously extracted with ether under nitrogen. The combined extract is dried over magnesium sulfate-sodium carbonate and evaporated under nitrogen. The residual oil is fractionally distilled in a microdistillation apparatus in a stream of nitrogen. The yield based on the nitrile is 65–75%, b.p. 130–140° (a redistilled authentic sample shows the same boiling range under the same conditions). A similar synthesis has been reported by Gauthier[1] with the ethyl Grignard reagent.

[1]D. Gauthier, Compt. rend., *152*, 1100 (1911).

2,3-BUTANEDIONE-1-C^{14}

$$(CH_3)_2CO + C^*H_3I \xrightarrow{h\nu} C^*H_3COCOCH_3 + CO + C^*H_4 + C^*H_3C^*H_3 + C^*H_3COCH_3$$

S. N. Naldrett, Can. J. Chem., *33*, 750 (1955).

A. Procedure (Note 1)

A mixture of 5.6 mmoles of acetone and 0.023 mmoles of iodomethane-C^{14} is irradiated at room temperature in a quartz cell with light of 2537 A° for 690 hours (Note 2). Product separation is accomplished by low-pressure fractionation in a Ward-Savelli still,[1] giving the following fractions (at the respective condenser temperatures): −183°, methane-C^{14} and carbon monoxide (Note 3); −140°, ethane-C_2^{14}; −78°, 2-propanone-1-C^{14}; and −60°, 2,3-butanedione-1-C^{14} (yield 0.38 mmoles, 8.7% radiochemical yield). Purification (Note 4) is accomplished by the addition of 0.75 g. of carrier 2,3-butanedione and 0.3 g. of acetone, separation of the acetone fraction in the still, and repetition of the fractionation process for a total of 4 times (Note 5).

B. Notes

1. The preparation is incidental to a study of the mechanism of photolysis of acetone.[2] The quantum efficiency appears to be close to unity for the production of methyl and acetyl free radicals, but their recombination to form acetone accounts for the low over-all quantum efficiency of decomposition of acetone at low temperatures. The presence of biacetyl does not seem to have any particular effect on the decomposition of acetone.

2. The cylindrical vessel (45 mm. × 108 mm.), having polished plane parallel windows, is illuminated by a neon-mercury resonance lamp (current 100 ma.) in a quartz envelope with a filter of chlorine gas. The light absorption in the cell is of the order of 10^{19} quanta/hour. A cooling tube at 0° serves to reduce the amount of biacetyl vapor in the light beam, thus reducing its reaction to carbon monoxide and ethane-C_2^{14}.

3. Carbon monoxide may be removed from methane-C^{14} by oxidation over hot copper oxide and absorption in Ascarite.

4. For the present purpose the presence of acetone in the product is not objectionable, provided it is nonisotopic.

5. No changes in the specific activity of the acetone and product fractions are obtained by further fractionation. Mass spectrometric analysis shows that the product contains about 14% nonisotopic acetone.

[1]J. J. Savelli, W. D. Seyfried and B. M. Filbert, Ind. Eng. Chem., Anal. Ed., *13*, 868 (1941).

[2]L. M. Dorfman and W. A. Noyes, Jr., J. Chem. Phys., *16*, 557 (1948); E. W. R. Steacie, *Atomic and Free Radical Reactions*, 2nd Ed., Reinhold, New York, 1954; H. W. Anderson and G. K. Rollefson, J. Am. Chem. Soc., *63*, 816 (1941).

2-PENTANONE-1-C^{14}

$$CH_3CH_2CH_2CN \xrightarrow{C^*H_3MgI} CH_3CH_2CH_2C(=NMgI)C^*H_3 \xrightarrow{H_2O} CH_3CH_2CH_2COC^*H_3$$

E. Cerwonka, R. C. Anderson and E. V. Brown, J. Am. Chem. Soc., 75, 28 (1953).

A. Procedure (Note 1)

The apparatus consists of a 250-ml. three-necked flask equipped with a mercury-sealed stirrer, a pressure-equalizing dropping funnel and a low-temperature condenser, which is cooled with Dry Ice-trichloroethylene and fitted with a drying tube. To a stirred mixture of 1.7 g. (71 mmoles) of dry magnesium turnings and 120 ml. of absolute ether is added slowly 0.33 g. (66 mmoles) of methyl-C^{14} iodide in an equal volume of ether. To

the stirred Grignard reagent (Note 2) is slowly added 4.0 g. (58 mmoles) of freshly distilled butyronitrile dissolved in an equal volume of absolute ether. The mixture is stirred for 3 hours and then hydrolyzed at −15° by addition of 100 ml. of 20% aqueous ammonium chloride solution, followed by 20 ml. of 6 *N* hydrochloric acid. The mixture is stirred at room temperature for 1 hour; then the ether phase is separated and fractionally distilled; yield 1.9 g. (33%), b.p. 100–102°.

B. Notes

1. The procedure is a modification of that of Hauser and Humphlett.[1]

2. Assay of an aliquot of the Grignard reagent, by the titration procedure of Gilman, Zoellner and Dickey,[2] shows the yield to be 64 mmoles, 97%.

C. Other Preparations

2-Heptanone-1-C^{14} is prepared (36% yield) by the same procedure, starting with hexanenitrile and methylmagnesium-C^{14} iodide.

[1]C. R. Hauser and W. J. Humphlett, J. Org. Chem., *15*, 359 (1950).

[2]H. Gilman, E. A. Zoellner and J. B. Dickey, J. Am. Chem. Soc., *51*, 1576 (1929).

3-PENTANONE-3-C^{13}
(Ethyl Ketone-C^{13})

$$(CH_3CH_2C^*OO)_2Ba \xrightarrow{\Delta} (CH_3CH_2)_2C^*O$$

H. G. Wood, C. H. Werkman, A. Hemingway, A. O. Nier and C. G. Stuckwish, J. Am. Chem. Soc., *63*, 2140 (1941).

A. Procedure

Barium propionate-1-C^{13} in a 25-ml. distilling flask is heated at 460° (Note 1) for 1 hour in a slow stream of dry nitrogen, and the product is collected in 45 ml. of ice-water in a 22-mm. test tube. The yield is 64% (0.95 mmole) as determined by precipitating the 2,4-dinitrophenylhydrazone, m.p. 155°, from an aliquot (Note 2).

B. Notes

1. There is virtually no decarboxylation at 350° and the yield is only 18% at 405°. Lukovnikov,[1] however, has described the preparation of 3-pantanone-3-C^{14} by pyrolysis of the barium salt at 350–370° in a con-

tinuous stream of nitrogen with the use of liquid nitrogen-cooled traps to collect the product (b.p. 101–103°, n_D^{25} 1.3911). The recovered barium carbonate-C^{14} contained one-half of the initial isotope.

2. Both the product and the derivative contain the expected isotopic concentration.

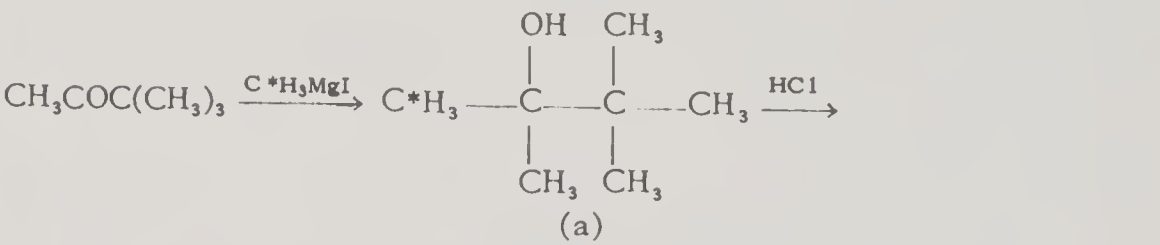
C. Other Preparations

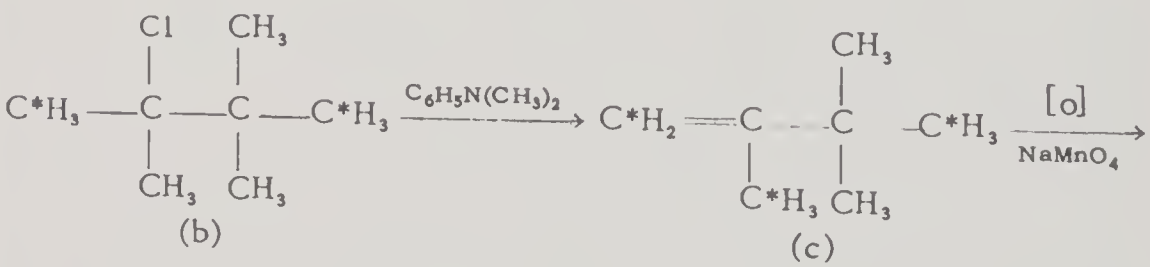
3-Pentanone-3-C^{14} and 3-pentanone-2,4-C_2^{14} have been prepared[2] in a similar manner from the corresponding propionates, and purified by low-pressure fractional distillation.

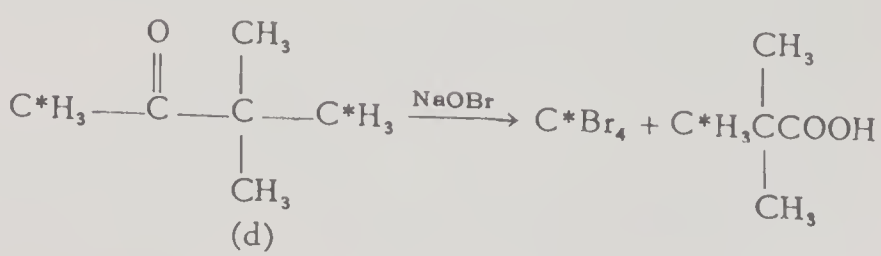
[1]A. L. Lukovnikov, M. B. Neyman, A. A. Bag, L. M. Rodionova, I. S. Samoukina and N. V. Bliznyak, Doklady Akad. Nauk S.S.S.R., *88*, 297 (1953); Nuc. Sci. Abstracts 7, 3054 (1953); AEC-tr-1829.

[2]A. Finkelstein and W. A. Noyes, Jr., Discussions Faraday Soc., *14*, 76 (1953).

3,3-DIMETHYL-2-BUTANONE-1,4-$C^{14}_{1/2}$
($C^{14}_{1/2}$-Pinacolone)







J. D. Roberts and J. A. Yancey, J. Am. Chem. Soc., 77, 5558 (1955).

A. Procedure (Note 1)

(a) *2,3,3-Trimethyl-2-butanol-1-C^{14}*. To a stirred Grignard solution, prepared from 40 g. of methyl-C^{14} iodide and 7 g. of magnesium in 160 ml. of absolute ether under nitrogen, is added dropwise 24 g. of pinacolone in 70 ml. of ether during 3 hours. The mixture is refluxed for 1 hour and decomposed with saturated ammonium chloride-ice. The aqueous layer is extracted with four 20 ml. portions of ether, and the combined extract is dried over magnesium sulfate. The ether is replaced with dry benzene (70 ml.), and the solution is refluxed for 3 hours over 45 g. of barium oxide. The product is diluted with 14.2 g. of carrier and distilled through a dried, 60 × 1-cm., helix-packed column; yield 88.5 g. (74%), b.p. 129–131° (Note 2). Degradation studies (Note 3) indicate that there is less than 6% rearrangement.

(b) *2-Chloro-2,3,3-trimethylbutane-1,4-$C^{14}_{1/2}$*. A mixture of 4.1 g. of 2,3,-3-trimethyl-2-butanol-1-C^{14}, 10 ml. of methylene chloride and 50 ml. of concentrated hydrochloric acid is stirred at 25° for 20 minutes. The aqueous layer is extracted with methylene chloride, and the combined extract is dried over magnesium sulfate and concentrated. The product is vacuum-distilled; yield 4.5 g., m.p. 130.2–132.6° (sealed tube). Degradation studies (see below) indicate that 34–37% of the isotope is located at C-4 (Note 4).

(c) *2-Methyl-$C^{14}_{1/2}$-3,3-dimethyl-1-butene-1,4-$C^{14}_{1/2}$*. A mixture of 5.0 g. of 2-chloro-2,3,3-trimethylbutane-1,4-$C^{14}_{1/2}$ and 40 ml. of *N,N*-dimethylaniline is refluxed for 1.75 hours and distilled through a semimicro column;[1] yield 3.2 g. (88%), b.p. 78°.

(d) *3,3-Dimethyl-2-butanone-1,4-$C^{14}_{1/2}$*. A mixture of 2.6 g. of the alkene and 17 g. of sodium permanganate trihydrate in 170 ml. of water, maintained at 10–20°, is shaken for 1.5 hours. Sulfur dioxide is bubbled through the ice-cooled mixture to destroy the manganese dioxide and excess reagent. The solution is extracted with five 20-ml. portions of ether, and the extract is dried over magnesium sulfate and concentrated beneath a short column. The product is distilled through a semimicro column;[1] yield 0.84 g. (33%), b.p. 102–106° (Note 5). Degradation studies (Note 6) indicate that the isotope is distributed among the methyl groups, with 36% of the activity (Note 4) located at a C-3 methyl group (C-4) as a result of rearrangement.

B. Notes

1. These preparations were incidental to a tracer study of the mechanism of carbonium-ion type reactions.

2. The alcohol must be protected rigorously from moisture, as it readily forms a hydrate, m.p. 80.9–81.2°.

3. A mixture of 4.8 g. of the alcohol, 14 g. of phenyl isocyanate and 2–4 drops of pyridine is refluxed for 4 hours at 150° and distilled. The fraction boiling below 90° is washed with three 10-ml. portions of cold water, dried over calcium chloride and distilled over barium oxide through a semimicro column;[1] yield 2.6 g. of 2-methyl-C^{14}_1-3,3-dimethyl-1-butene-1-C^{14}_1 (62%), b.p. 78°. A sample absorbs 99.2% of the calculated amount of hydrogen over platinum oxide. Further degradation indicates that there is only 3–6% isotopic rearrangement, in contrast with preparation (c) which has 36% rearrangement.

4. The rearrangement is 59% (essentially statistical, i.e. 60:40) when the reaction is carried out with 6.0 ml. of concentrated hydrochloric acid and 9.60 g. of anhydrous zinc chloride. Thus this rearrangement does not involve a symmetrical non-classical cationic intermediate (with equivalent carbons at 2 and 3) in an irreversible carbonium ion type reaction (50:50 distribution). Under the same conditions the chloride is completely equilibrated with the Lucas reagent containing chloride-Cl^{36}.

Although the system is labile, the extent of rearrangement may be greatly reduced by working under mild conditions. Treatment of the alcohol with concentrated hydrochloric acid, at 0° for 1 minute, gives only 12–16% rearrangement and 1% halogen exchange. There is no rearrangement in neutral hydrolysis of the chloride in water, where all the steps are likely to be irreversible.

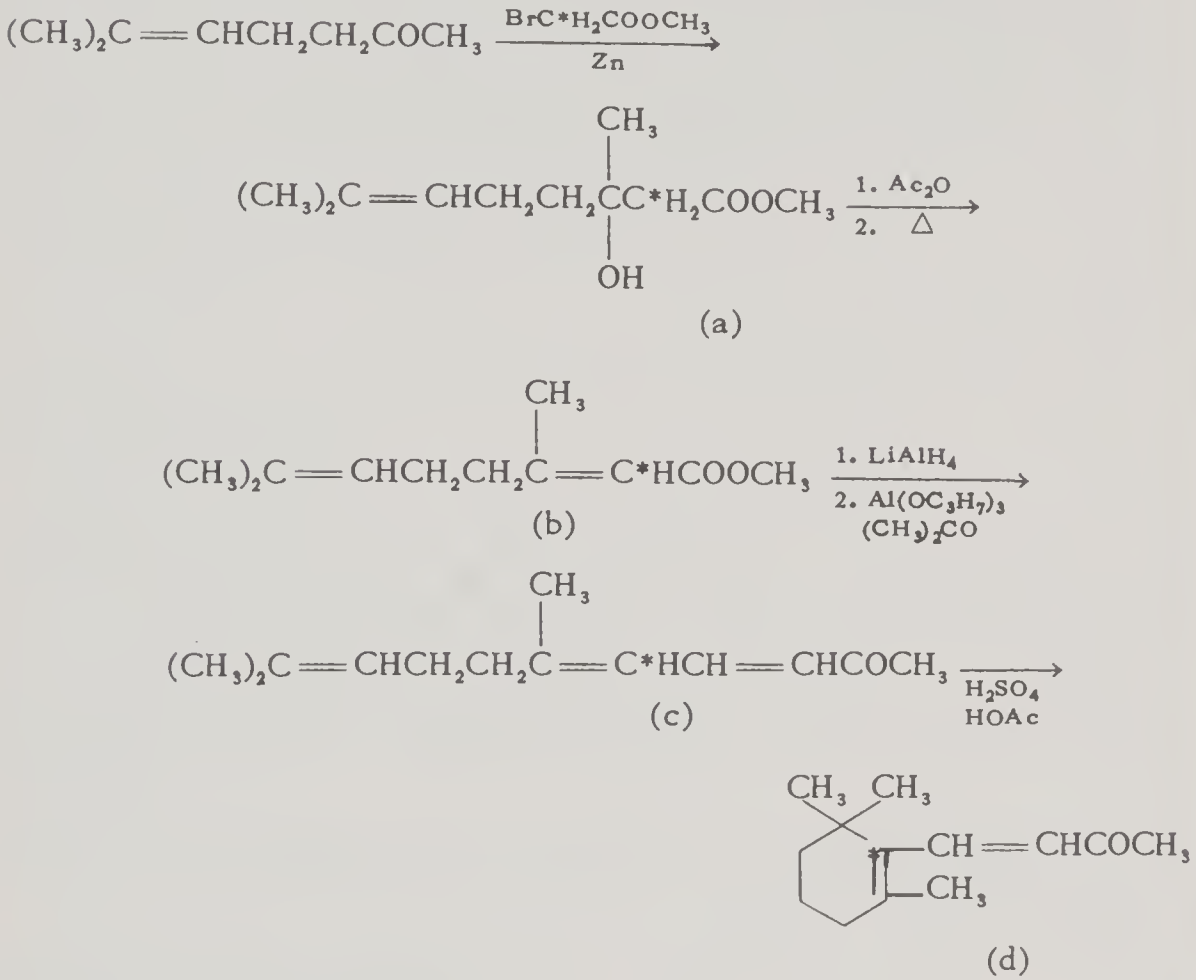
5. The compound is assayed as the 2,4-dinitrophenylhydrazone, m.p. 127°. The molar specific activity is about 63.5% of that of the starting material.

6. A mixture of 1.0 g. of the $C^{14}_{1/2}$-pinacolone, 1.8 ml. of bromine, 12 g. of sodium hydroxide and 40 ml. of water is stirred for 3 hours at 0–10°. The precipitate is steam-distilled, collected, and sublimed twice, yielding 1.5 g. (46%) of carbon-C^{14} tetrabromide, m.p. 95°. The molar specific activity is nearly equal to the difference between those of the olefin and ketone, being 27.7% and 43.7% of these, respectively.

The residue from the steam distillation is acidified with concentrated sulfuric acid, then treated with 13 g. of silver sulfate and again steam-distilled. The distillate is titrated with sodium hydroxide and evaporated, yielding sodium 2,2-dimethylpropionate-3-C^{14}, which is assayed as *p*-bromophenacyl 2,2-dimethylpropionate-3-C^{14}, m.p. 77.0–77.4° from aqueous ethanol. The molar specific activity is 34.0% of that of the olefin, and 53.5% of that of the ketone.

[1]C. W. Gould, G. Holzman and C. Niemann, Anal. Chem., *20*, 361 (1948).

4-(2,6,6-TRIMETHYL-1-CYCLOHEXEN-1-YL-1-C^{14})-3-BUTEN-2-ONE (C_1^{14}-β-Ionone)



B. G. Engel, J. Würsch and M. Zimmermann, Helv. Chim. Acta, 36, 1771 (1953).

A. Procedure

(a) *Methyl 3-Hydroxy-3,7-dimethyl-6-octenoate-2-C^{14}* (Note 1). The crude methyl bromoacetate-2-C^{14} prepared from 0.091 g. of bromoacetic-2-C^{14} acid, by esterification with diazomethane, is mixed with 4.0 g. of methyl bromoacetate, 4.1 g. of freshly distilled 6-methyl-5-hepten-2-one, 2.3 g. of iodine-activated zinc chips and 8 ml. of dry benzene. The mixture is refluxed for 30 minutes, then poured onto ice and extracted with ether. The extract is washed with dilute sulfuric acid, sodium carbonate solution and water, then dried over sodium sulfate and concentrated, and the product is distilled; yield 3.3 g., b.p. 119–120° (12 mm.) (Note 2). The forerun is reprocessed with 1.0 g. of methyl bromoacetate, 1.5 g. of 6-methyl-5-hepten-2-one, 1.3 g. of zinc and 5 ml. of benzene. The yield is 1.1 g., b.p. 115°, making the total yield of ester 4.4 g. (Note 3), 66% based on 5.1 g. of methyl bromoacetate.

(b) *Methyl Geranate-2-C^{14}*, (*Methyl 3,7-Dimethyl-2,6-octadienoate-2-C^{14}*) (Note 4). A mixture of 5.2 g. of methyl 3-hydroxy-3,7-dimethyl-6-octenoate-2-C^{14} and 15 ml. of distilled acetic anhydride is refluxed for 8 hours, then concentrated under vacuum on a steam-bath. The crude methyl 3-acetoxy-3,7-dimethyl-6-octenoate-2-C^{14} is pyrolyzed by dropwise addition to a flask heated at 320–330°, the crude product being distilled through a 9-cm. Vigreux column. The acetic acid is removed and the product is distilled; yield 3.7 g. (78%), b.p. 98–104° (10 mm.) (Note 5). The apparatus is scavanged with 3.0 g. of carrier, making the total yield 6.7 g.

(c) *6,10-Dimethyl-3,5,9-undecatrien-2-one-5-C^{14}*, (*C_1^{14}-Pseudoionone*) (Note 6). To a solution of 1.1 g. of lithium aluminum hydride (50% excess) in 50 ml. of absolute ether at room temperature is added dropwise 6.7 g. of methyl geranate-2-C^{14} in 15 ml. of ether. The mixture is refluxed for 45 minutes, then hydrolyzed and extracted.

A solution of the crude geraniol-2-C^{14} in 220 ml. of absolute benzene is dried azeotropically by distillation of 30 ml., then is mixed with 80 ml. of anhydrous acetone and 12.0 g. of aluminum isopropoxide (Note 7). The mixture is protected from air and moisture, and refluxed for 41 hours. The solvent is removed through a column, and the mixture is hydrolyzed in the cold with dilute hydrochloric acid and extracted with ether. The extract is washed with water and sodium carbonate, then dried, concentrated and distilled; yield 3.68 g. of C_1^{14}-pseudoionone, b.p. 66–81° (10 μ) (Note 8). The distillation forerun (1.53 g.) is reprocessed with acetone and aluminum isopropoxide, yielding 1.03 g. of product, b.p. 72–85° (70 μ). The total yield is 4.71 g., 66% based on methyl geranate (Note 9).

(d) *4-(2,6,6-Trimethyl-1-cyclohexen-1-yl-1-C^{14})-3-buten-2-one*, (*C_1^{14}-β-Ionone*) (Note 10). The C_1^{14}-pseudoionone (6.91 g.) is cyclized in two portions by dropwise addition to a stirred mixture of concentrated sulfuric and glacial acetic acids (7 : 3 by weight) at room temperature. The mixture is stirred for about 25 minutes, then poured into water, and the product is extracted with ether, dried and distilled; yield 4.59 g. (66.5%), b.p. 66–69° (40 μ) (Note 11). The product contains about 20% C_1^{14}-α-ionone (Note 12). The over-all yield is 23% based on bromoacetic acid.

B. Notes

1. The procedure is a modification of that of Ruzicka.[1]
2. The product from trial runs boiled at 120–122° (10 mm.).
3. The distillation flask is scavanged with 0.8 g. of carrier ester, making the final yield 5.2 g.
4. The method is that of Favre.[2]
5. Trial preparations boiled at 100–102° (11 mm.).
6. The reduction is by the method described by Favre,[2] and the simultaneous oxidation and condensation are after the method of Schinz.[3]

7. The preparation of reagent is described by Wilds.[4]

8. The product from a trial preparation boiled at 84° (20 μ). Pure pseudoionone boils at 143–145° (12 mm.), n_D^{20} 1.5275.[5]

9. The apparatus is scavanged by distilling 1.5-g. and 0.7-g. portions of carrier, making the final yield 6.91 g.

10. The procedure is an adaptation of that of Royals.[5] Ring closure is brought about with acid.[5,6] With strong acids the main product is β-ionone (b.p. 127–128.5° at 10 mm.), while with weak acids the chief product is α-ionone or 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one (b.p. 123–124° at 11 mm.).

11. The product from a trial run boiled at 67–68° (50 μ). The total yield is raised to 6.29 g. by scavanging the apparatus with 1.7 g. of carrier.

12. The large-scale preparations give 71.5% yields of product containing 90% β-isomer on the basis of refractive index determination: α-ionone, n_D^{20} 1.4980; β-ionone, n_D^{17} 1.521.

[1]L. Ruzicka and H. Schinz, Helv. Chim. Acta, *23*, 965 (1940); *Organic Reactions*, Vol. I, Wiley, New York, 1942, Chapt. 1.

[2]H. Favre and H. Schinz, Helv. Chim. Acta, *35*, 1632 (1952).

[3]H. Schinz, L. Ruzicka, C. F. Seidel and Ch. Tavel, Helv. Chim. Acta, *30*, 1810 (1947); *Organic Reactions*, Vol VI, Wiley, New York, 1951, Chapt. 5.

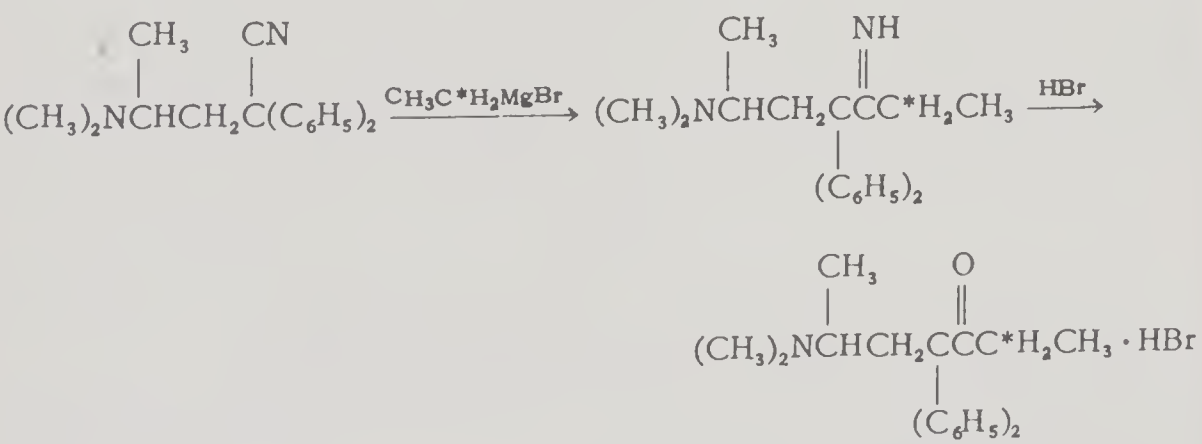
[4]*Organic Reactions*, Vol. II, Wiley, New York, 1944, Chapt. 5.

[5]E. E. Royals, Ind. Eng. Chem., *38*, 546 (1946).

[6]H. Hibbert and L. T. Cannon, J. Am. Chem. Soc., *46*, 119 (1924).

6-DIMETHYLAMINO-4,4-DIPHENYL-3-HEPTANONE-2-C^{14} HYDROBROMIDE

(C_1^{14}-Methadone Hydrobromide)



B. M. Tolbert, F. Christenson, F. N. H. Chang and P. P .T. Sah, J. Org. Chem., *14*, 525 (1949).

A. Procedure (Note 1)

The apparatus consists of a small pear-shaped flask attached to a vacuum line and equipped with an induction stirrer, a low-temperature condenser cooled with Dry Ice-acetone, and an addition funnel (Note 2). To a mixture of 0.35 g. of magnesium turnings and 10 ml. of absolute ether, frozen with liquid nitrogen, is added 1.43 g. (13.1 mmoles) of ethyl-1-C^{14} bromide by vacuum distillation. The flask is closed off from the manifold, and the stirred mixture is refluxed for 1 hour. To the Grignard solution is added slowly 2.35 g. of 4-dimethylamino-2,2-diphenylvaleronitrile (Note 3) in 6 ml. of dry benzene, rinsing the funnel with two 1-ml. portions of benzene (Note 4). The mixture is refluxed for 3 hours; then the complex is cooled to 0° and decomposed by the dropwise addition of 24.4 ml. of 20% hydrobromic acid. The volatile solvents are distilled off on a steam-bath, and the cooled aqueous solution is washed with ether, then made alkaline with 20 ml. of 3 *N* sodium hydroxide and extracted with ether. The extract is concentrated to small volume, acidified with 2 ml. of 20% hydrobromic acid, heated to expel ether and treated with a few ml. of absolute alcohol. The solution is concentrated on the steam-bath, and the crystalline product is collected and recrystallized from 80% alcohol; yield 1.00 g. (19.5%), m.p. 224° (Note 5).

B. Notes

1. The procedure is adapted from the method outlined by Schultz.[1]

2. A diagram of the apparatus is presented by Tolbert.

3. The nitrile is prepared according to Schultz,[1] m.p. 91–91.2°, and dried under vacuum over calcium chloride.

4. Care is taken to exclude air from the reaction flask.

5. The free base melts at 78°, and the hydrochloride melts at 235–236°. A high order of purity was established[2] by countercurrent distribution technique. A determination of methadone is described by Way.[3]

C. Other Preparations

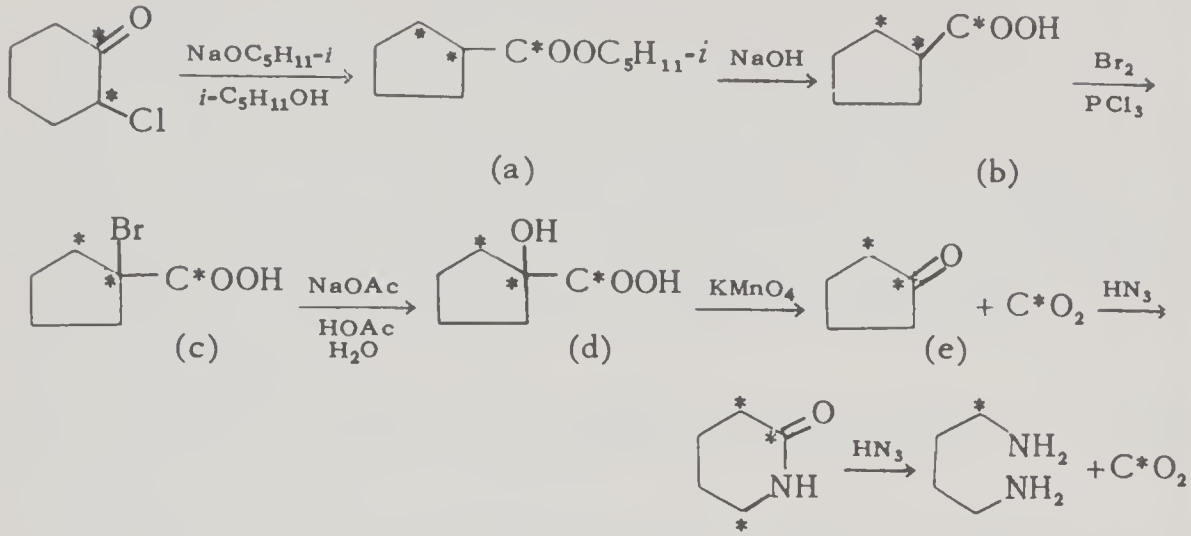
In the same manner from ethyl-2-C^{14} bromide is prepared 6-dimethylamino-4,4-diphenyl-3-heptanone-1-C^{14} hydrobromide.

[1]E. M. Schultz, C. M. Robb and J. M. Sprague, J. Am. Chem. Soc., 69, 188, 2454 (1947).

[2]E. L. Way, B. T. Signorotti, C. H. March and C. T. Peng, J. Pharm. Exp. Therap., *101*, 249 (1951).

[3]E. L. Way, C. Sung and W. P. McKelway, J. Pharm. Exp. Therap., 97, 222 (1949).

CYCLOPENTANONE-1,2-$C^{14}_{1/2}$



R. B. Loftfield, J. Am. Chem. Soc., 73, 4707 (1951); 72, 632 (1950).

A. Procedure (Note 1)

(a) *Isopentyl Cyclopentanecarboxylate-$C^{14}_{1/1}$-1,2-$C^{14}_{1/2}$.* To a solution of 1.8 g. (78 mmoles) of sodium in 44 ml. of isoamyl alcohol at −5° is added a solution of 13.8 g. (102 mmoles) of 2-chlorocyclohexanone-1,2-$C^{14}_{1/2}$ in 10 ml. of isoamyl alcohol (Note 2), then the mixture is left in a refrigerator overnight. The mixture is extracted with 20 ml. of water and the organic layer is separated, filtered, and fractionally distilled; yield 6.80 g., b.p. 108–109° (14 mm.) (Note 3).

(b) *Cyclopentanecarboxylic-$C^{14}_{1/1}$-1,2-$C^{14}_{1/2}$ Acid.* A mixture of 5.4 g. of isopentyl cyclopentanecarboxylate-$C^{14}_{1/1}$-1,2-$C^{14}_{1/2}$, 10 ml. of alcohol, 5 ml. of water and 1.5 g. of sodium hydroxide is refluxed for 3 hours. The mixture is acidified with hydrochloric acid, and the product is extracted into ether and distilled; yield 2.50 g., b.p. 103–105° (8 mm.).

(c) *1-Bromocyclopentanecarboxylic-$C^{14}_{1/1}$-1,2-$C^{14}_{1/2}$ Acid.* A mixture of 2.30 g. of cyclopentanecarboxylic-$C^{14}_{1/1}$-1,2-$C^{14}_{1/2}$ acid, 1.10 ml. of bromine and 0.20 g. of phosphorus trichloride is heated at 100° in a sealed tube for 1 hour. The tube is opened and most of the hydrogen bromide and excess bromine are removed by evaporation at 50°, under the reduced pressure of a water-pump. The yield is nearly quantitative.

(d) *1-Hydroxycyclopentanecarboxylic-$C^{14}_{1/1}$-1,2-$C^{14}_{1/2}$ Acid.* The crude 1-bromocyclopentanecarboxylic-$C^{14}_{1/1}$-1,2-$C^{14}_{1/2}$ acid is dissolved in a solution of 30 ml. of acetone, 30 ml. of water, 2.5 ml. of acetic acid and 2.0 g. of sodium acetate, then set aside at room temperature for 16 hours (Note 4). The acetone is removed by distillation; the residue is adjusted to pH 2 with hydrochloric acid and extracted continuously with ether. The extract is evaporated to dryness, and the residue is dissolved in ethyl

acetate and decolorized with charcoal. Dilution with petroleum ether gives 1.60 g. of product, m.p. 105.5–106° (Note 4).

(e) *Cyclopentanone-1,2-$C^{14}_{1/2}$.* A mixture of 0.813 g. of 1-hydroxycyclopentanecarboxylic-$C^{14}_{1/1}$-1,2-$C^{14}_{1/2}$ acid and 0.020 g. of potassium permanganate in 5 ml. of water is heated in a distillation flask at 50° while a stream of nitrogen carries the evolved carbon-C^{14} dioxide into an alkali trap. The oxidation is continued with addition of another 20 mg. of potassium permanganate and collection of a second sample of carbon-C^{14} dioxide (Note 5). A mixture of 1.5 g. of phthalic acid, 0.600 g. of 1-hydroxycyclopentanecarboxylic-$C^{14}_{1/1}$-1,2-$C^{14}_{1/2}$ acid and 40 ml. of water is added, and the mixture is distilled while a solution of 0.700 g. of potassium permanganate in 20 ml. of water is added dropwise. A total of 5.4 ml. of distillate is collected (Note 6); the molar specific activity of the product is 48–51% of that of the starting material. Degradation studies indicate that the isotope is equally distributed as indicated (Note 7).

B. Notes

1. These preparations were incidental to a study of the mechanism of the Faworskii reaction.

2. These conditions are those of the Faworskii reaction[1] in which α-haloketones are rearranged by base to carboxylic acids (esters or amides) of the same number of carbon atoms. Salt precipitates almost at once, and the mixture warms spontaneously to about 40°.

3. Since only 77% of the theoretical amount of base is employed in this case, 1.60 g. of 2-chlorocyclohexanone-1,2-$C^{14}_{1/2}$, b.p. 89–90° (14 mm.), is recovered of 48% purity on the basis of infrared analysis, isotope dilution technique and conversion to the osazone. Degradation studies (see 2-hydroxycyclohexanone-1,2-$C^{14}_{1/2}$) indicate that there is no isotopic rearrangement (or halogen migration) of the starting material under these conditions which could otherwise explain the α,β randomization of the Faworskii rearrangement. The yield of cyclopentanecarboxylic acid is 65% (b.p. 117–118° at 25 mm.), when stoichiometric amounts of sodium ethoxide and chloroketone are reacted in ethanol at 0°, but becomes very low when the bromoketone is employed under the same conditions.

4. The elimination of HBr is minimized by hydrolysis of the α-bromo carboxylate[2] anion under acidic conditions.

5. The carbon dioxide contains 49–52% of the initial molar specific activity and represents the carbonyl group of the unrearranged α-chloroketone.

6. The dinitrophenylhydrazone melts at 146–147°, and the semicarbazone melts at 207–208°.

7. Directions are given in the original paper for converting the ketone to 5-aminovaleric acid hydrochloride by the Schmidt reaction.[3] This material is then decarboxylated, by another Schmidt reaction, forming C_1^{14}-putrescine hydrochloride which contains 24–25% of the initial specific activity, while the evolved carbon-C^{14} dioxide accounts for a like amount, representing the C-2 and C-1, respectively, of the cyclopentanecarboxylic acid.

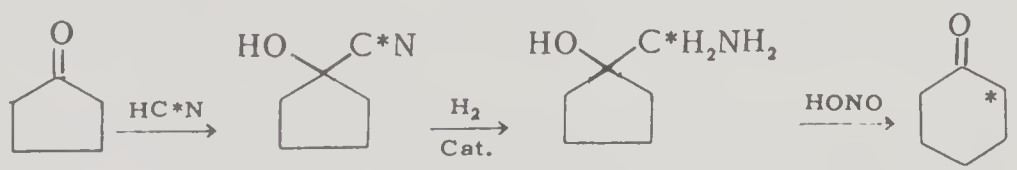
C. Other Preparations

By the same series of reactions, starting with 2-chlorocyclohexanone-1-C^{14}, are prepared: isopentyl cyclopentanecarboxylate-C^{14}, cyclopentanecarboxylic-C^{14} acid, 1-bromocyclopentanecarboxylic-C^{14} acid, and 1-hydroxycyclopentanecarboxylic-C^{14} acid. Permanganate oxidation of the hydroxy acid forms carbon-C^{14} dioxide and nonisotopic cyclopentanone. Thus, it is proven that during the Faworskii rearrangement the carbonyl carbon becomes the new carboxyl carbon without randomization.

Starting with cyclopentanecarboxylic-1-C^{14} acid, the same series of reactions produce: 1-bromocyclopentanecarboxylic-1-C^{14} acid, 1-hydroxycyclopentanecarboxylic-1-C^{14} acid, and cyclopentanone-1-C^{14}. The evolved carbon dioxide is nonisotopic. Degradation of the cyclopentanone-1-C^{14} yields nonisotopic putrescine and carbon-C^{14} dioxide of unchanged specific activity. As this carbon dioxide originates as the carbon of the carboxylic acid, one now is able to distinguish among the carbons of the carbonyl, 1 and 2 positions of the product of the Faworskii rearrangement. A randomization process leads to equal radioactivities of the α and β positions. These observations have discredited five proposed mechanisms[4] for the rearrangement.

[1]R. Jacquier, Bull. soc .chim. France, Documentation, *1950*, D35.
[2]E. Grunwald and S. Winstein, J. Am. Chem. Soc., *70*, 841 (1948).
[3]*Organic Reactions*, Vol. III, Wiley, New York, 1946, p. 335.
[4]R. B. Loftfield, J. Am. Chem. Soc., *72*, 632 (1950); *73*, 4707 (1951).

CYCLOHEXANONE-2-C^{14}



(a) (b)

R. T. Arnold, Atomic Energy Commission Report, AECU-575; Nuc. Sci. Abstracts, *3*, 2137 (1949).

A. Procedure (Note 1)

(a) *1-Hydroxycyclopentanecarbonitrile-C^{14}*, (*Cyclopentanone Cyanohydrin-C^{14}*) (Note 2). To a stirred mixture of 2.77 g. (41 mmoles) of potassium cyanide-C^{14}, 5 ml. of water, 15 ml. of ether and 3.4 g. (40.4 mmoles) of cyclopentanone, maintained at 0° to −5°, is added 4.5 g. of 90% formic acid dropwise over a period of 2 hours. The mixture is stirred for 4 additional hours at 0° and stored in a refrigerator for 2 days. The product is extracted from the cold equilibrium mixture with ether, and the extract is washed with water and concentrated under a column on a steam-bath.

(b) *Cyclohexanone-2-C^{14}*. The crude cyanohydrin, dissolved in 50 ml. of alcohol and 20 ml. of acetic acid, is hydrogenated at 40 p.s.i. over platinum prepared from 0.40 g. of platinum oxide. If hydrogenation ceases short of the 80-mmole uptake (Note 3), the solution is filtered and the reaction is completed over excess Raney nickel (2 g.). The solution is filtered and treated with 3 ml. of acetic acid, and the alcohol is evaporated under diminished pressure. Water (50 ml.) is added, and the solution of crude 1-(aminomethyl-C^{14})cyclopentanol is cooled to 0° to −5° and treated with 3.0 g. of sodium nitrite in 5 ml. of water. After 20 minutes, the cold mixture is warmed to 70° for 1.5 hours, during which time nitrogen is evolved. The mixture is extracted with two 100-ml. portions of ether, and the extract is washed with 10% potassium bicarbonate solution, dried over sodium sulfate and concentrated. The residual oil is distilled up to 80° at 1 mm. with the distillate (1.5 ml.) being collected in a Dry Ice trap. The product is collected by redistillation at atmospheric pressure, b.p. 145–162° (bath temperature); yield 0.989 g., 20–25% based on cyclopentanone (Note 5). Degradation studies indicate that there is no isotopic rearrangement and that the purity of the cyclohexanone-2-C^{14} is at least 85% (Note 6).

B. Notes

1. The method is adapted from the method of ring expansion described by Tiffeneau.[1] An amino alcohol, in which primary amino groups and hydroxyl groups are attached to adjacent carbon atoms, may undergo a Demjanow or semipinacolone rearrangement under the influence of nitrous acid, forming a carbonyl compound with a change in ring size; also see Ruzicka.[2]

2. The procedure is an adaptation of the method of Plattner,[3] which was found to be preferable to the direct addition of cyanide ion to the carbonyl bisulfite addition compound[4] (poisoned hydrogenation catalyst), or the addition of anhydrous hydrogen cyanide to the carbonyl compound

in the presence of cyanide ion as catalyst[4] (mechanical difficulties and variable results).

3. Hydrogen uptake ceased at one-third of theory. Addition of 0.20 g. of platinum oxide again gave evidence of catalyst poisoning. Complete reduction required 6.5 hours.

4. The yield of cyclohexanone was approximately 50% on large-scale trial runs, but always dropped with small runs.

5. The lower yield of cyclohexanone, compared to cycloheptanone, is attributed to the thermodynamically less favorable cyanohydrin formation[5] from the five-membered ring.

6. The ketone was oxidized to hexanedioic-1,2-$C^{14}_{1/2}$ acid (0.83 g., m.p. 145.5–147°) by stirring overnight with 3.2 g. of potassium permanganate in alkaline solution. The barium salt from 0.775 g. of the adipic acid was pyrolyzed (280–290°, 2.5 hours), and the distilled cyclopentanone-1,2-$C^{14}_{1/2}$ was caught in a Dry Ice trap, while the evolved carbon-C^{14} dioxide was trapped in sodium hydroxide and converted to barium carbonate-C^{14} (0.584 g.) for assay. The ketone was converted to the semicarbazone (0.431 g., m.p. 206–207°) for assay as barium carbonate-C^{14}. The cyclopentanone-1,2-$C^{14}_{1/2}$ contained 75% of the activity of the hexanedioic-1,2-$C^{14}_{1/2}$ acid, while the carbon dioxide contained 25%, as expected in the absence of any isotope effect.

The mechanism of the pyrolysis of barium hexanedioate-1-C^{13}, forming cyclopentanone-1-C^{13} and barium carbonate-C^{13}, is discussed by Bigeleisen.[6] A degradation of the cyclopentanone-1-C^{13} to 2,5-bis(*p*-dimethylaminobenzylidene)cyclopentanone-1-C^{13} (100% yield) and then carbon-C^{13} dioxide (60% yield) and succinic acid is described. There is little or no isotope effect in the irreversible pyrolytic decarboxylation (6 hours, 350°).

C. Other Preparations

By the same procedure, starting with cyclohexanone, is prepared cycloheptanone in 44.5% yield, b.p. 179–181°, n_D^{21} 1.4575. In contrast to cyclohexanone, the yield of cycloheptanone does not diminish with small-scale runs.

[1]M. Tiffeneau, P. Weill and B. Tchoubar, Compt. rend., *205*, 54 (1937); B. Tchoubar, Compt. rend., *212*, 195 (1941).

[2]L. Ruzicka, P. A. Plattner and H. Wild, Helv. Chim. Acta, *26*, 1631 (1943).

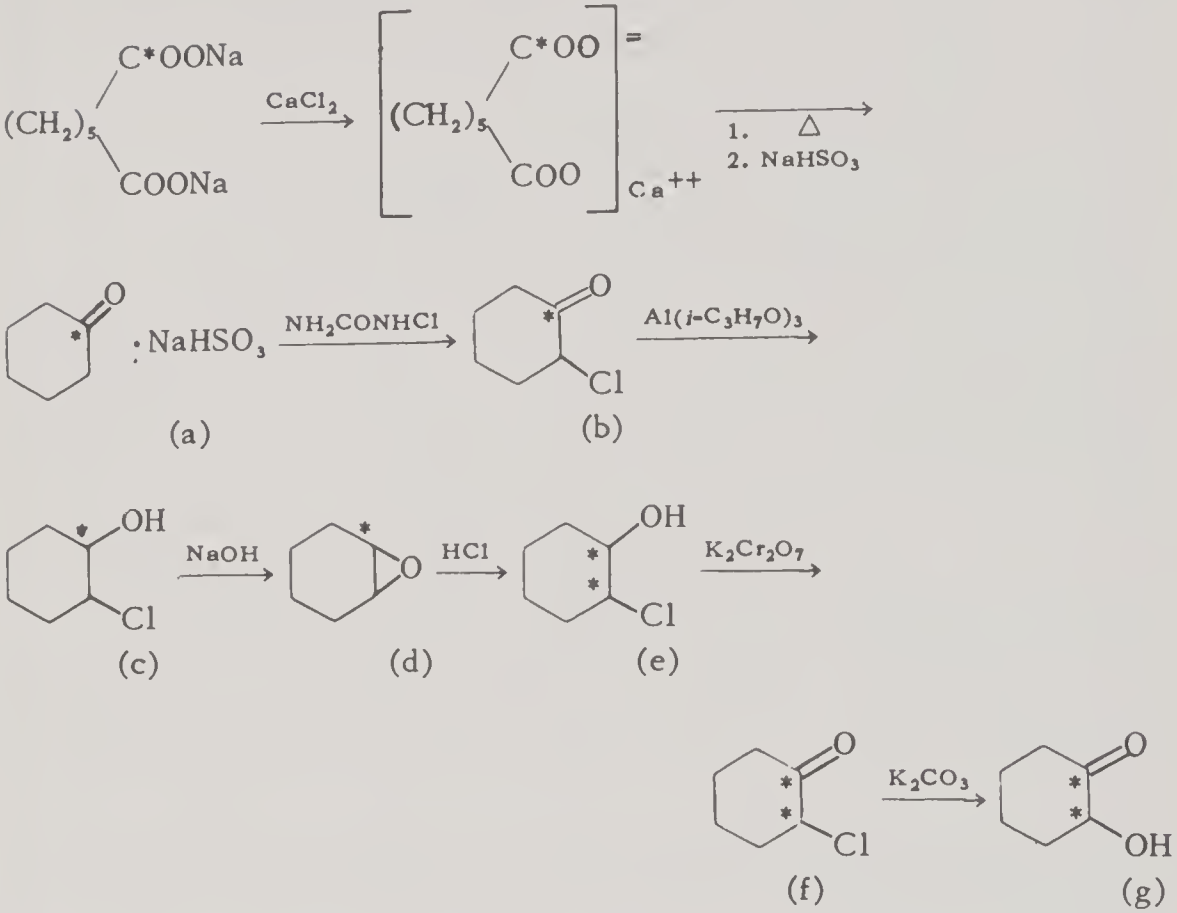
[3]P. A. Plattner, A. Furst and A. Studer, Helv. Chim. Acta, *30*, 1091 (1947).

[4]A. H. Cook and R. P. Linstead, J. Chem. Soc., *1934*, 959.

[5]A. Lapworth and R. H. F. Manske, J. Chem. Soc., *1928*, 2533; V. Prelog and M. Kobelt, Helv. Chim. Acta, *32*, 1187 (1949).

[6]J. Bigeleisen, A. A. Bothner-By and L. Friedman, J. Am. Chem. Soc., *75*, 2908 (1953).

2-HYDROXYCYCLOHEXANONE-1,2-$C^{14}_{1/2}$



R. B. Loftfield, J. Am. Chem. Soc., 73, 4707 (1951).

A. Procedure

(a) *Cyclohexanone-1-*C^{14}. To a solution of 3.20 g. (20 mmoles) of heptanedioic-1-C^{14} acid in 60 ml. of water, made just alkaline to phenolphthalein with sodium hydroxide, is added a solution of 5.0 g. (45 mmoles) of calcium chloride in 20 ml. of water. The mixture is concentrated to 30 ml. on a steam-bath and filtered. The yield of calcium heptanedioate-1-C^{14} is 4.60 g., after drying under vacuum over phosphorus pentoxide (Note 1). The salt, packed into a 10 × 200-mm. Pyrex tube with a 6-mm. delivery tube, is pyrolyzed by gradually heating to about 500° in an oven while the distillate is collected in a test tube chilled in ice water. A solution of 4.0 g. of sodium bisulfite in 6 ml. of water is added to the distillate, and the mixture is left in an ice-bath overnight. The cyclohexanone-1-C^{14} sodium bisulfite addition compound weighs 3.15 g., after being filtered off, washed with ether and dried (Note 2).

(b) *2-Chlorocyclohexanone-1-*C^{14}. The cyclohexanone-1-C^{14} sodium bisulfite compound (2.00 g.) is shaken with a mixture of 20 ml. of water, 5.0 g. of potassium carbonate and 20 g. of carrier cyclohexanone. The

organic phase is separated and stirred in a water-bath at 24° with a mixture of 10 ml. of acetic acid and 100 ml. (0.24 mole) of chlorourea solution (Note 3). When titration of aliquots indicates an abrupt change in the chlorination rate (5 hours), the product is extracted into 50 ml. of benzene, which is washed three times with water, dried over sodium sulfate, filtered and dried by brief boiling. Distillation gives a product yield of 22.0 g. (77%), b.p. 89–90° (14 mm.), m.p. 20–24° (Note 4).

(c) *2-Chlorocyclohexanol-1-C*^{14}. The dry benzene solution of crude 2-chlorocyclohexanone-1-C^{14} (without isolation) is added to a refluxing solution of 50 g. of aluminum isopropoxide in 700 ml. of absolute isopropyl alcohol[1]. After 12 minutes, the mixture is chilled in an ice-bath, and the solvent is removed by distillation at 15 mm. (Note 5). To the heavy syrup is added 30 ml. of concentrated hydrochloric acid and 200 g. of cracked ice. The product is extracted into ether, which is then washed with saturated sodium chloride solution, dried over sodium sulfate and distilled. The yield is 17.5 g. (60%), b.p. 78–82° (12 mm.) (Note 6).

(d) *7-Oxabicyclo[4.1.0]heptane-1-C*^{14}, (*Cyclohexene-1-C*^{14} *Oxide*). The 2-chlorocyclohexanol-1-C^{14} (3.3 g.) is diluted with 62.5 g. of carrier chlorohydrin (40.3 g. total *trans*-form) (Note 7), and stirred for 10 minutes with 16.1 g. of sodium hydroxide in 25 ml. of water and 60 ml. of ethanol. Potassium carbonate (20 g.) is added, the mixture is filtered, and the filtrate is distilled under reduced pressure. Redistillation from potassium hydroxide at atmospheric pressure[2] gives a yield of 20.4 g. (70%), b.p. 129–134° (Note 8).

(e) trans-*2-Chlorocyclohexanol-1,2-C*$^{14}_{1/2}$. A solution of the cyclohexene-1-C^{14} oxide in ether at −40° is saturated with anhydrous hydrogen chloride. The mixture is then warmed to room temperature, the solvent and excess reagent are removed at the water pump, and the residue is vacuum-distilled; yield 25.0 g. (89%), b.p. 86–87° (17 mm.), m.p. 26–28° (Note 9).

(f) *2-Chlorocyclohexanone-1,2-C*$^{14}_{1/2}$. To a vigorously stirred mixture of 25.0 g. of *trans*-2-chlorocyclohexanol-1,2-C$^{14}_{1/2}$, 18.0 g. of potassium dichromate and 15 ml. of water at 20–22° is added a solution of 25.0 g. of sulfuric acid and 15 ml. of water in 1-ml. portions at 10-minute intervals. Fifty ml. of water is added, and the mixture is stirred overnight at room temperature, then diluted with 200 ml. of water and 10 ml. of hydrochloric acid and extracted with two 75-ml. portions of benzene. The extract is combined, washed twice with water and once with saturated sodium chloride solution, then dried over sodium sulfate and distilled. The yield is 14.4 g. (58%), b.p. 69–71° (6 mm.), m.p. 18–20° (Note 10).

(g) *2-Hydroxycyclohexanone-1,2-C*$^{14}_{1/2}$. A mixture of 1.84 g. of 2-chlorocyclohexanone-1,2-C$^{14}_{1/2}$, 4 g. of potassium carbonate and 20 ml. of water is stirred overnight at room temperature.[3] Extraction with ether and recrystallization from alcohol yields 0.80 g. of product, m.p. 127–129°.

Degradation studies indicate that the isotope is distributed as indicated (Note 11).

B. Notes

1. The theoretical yield is 3.96 g.

2. Two additional crops of lower specific activity are obtained by treating the mother liquor with 1.0-g. portions of carrier cyclohexanone.

3. The chlorourea solution is prepared by absorbing 105 g. of chlorine in a stirred mixture of 150 ml. of water, 150 g. of calcium carbonate and 300 g. of urea. The solution is filtered through diatomaceous earth and diluted to 600 ml. with water. There is little polychlorination with this reagent,[4] since the product is insoluble and the rate of reaction is conveniently followed by iodometric titration of aliquots.

4. The recovery of starting materials is 2.0 g.

5. The long heating and slow distillation of acetone and solvent of the usual Meerwein-Ponndorf-Verley reduction[5] (cyclohexanol,[6] 95%) is detrimental in the case of certain haloketones.[1]

6. Titration with cold alkali, by the method of Bartlett,[7] indicates the presence of 54% of the *trans*-isomer.

7. The usual preparation[8] of 2-chlorocyclohexanol, by addition of hypochlorous acid to cyclohexene, when modified by the procedure of Bartlett,[7] leads to formation of the pure *trans*-isomer. Reduction of 2-chlorohexanone by Grignard reagents leads to a high percentage of the *cis*-isomer.

8. The rate of reaction with sodium hydroxide at 25° is 300 times greater for the *trans*- than for the *cis*-isomer and the respective products are the oxide and the ketone.

9. Titration [7] shows the absence of any *cis*-isomer.

10. The dinitrophenylosazone, m.p. 236–238°, forms in 45% yield. The infrared absorption spectrum corresponds to at least 90% purity. The distillation also gives a 3.1-g. fraction, b.p. 62–69° (6 mm.), m.p. 13–19°.

11. Directions are given by Loftfield for oxidizing the product with permanganate, forming hexanedioic-1-C^{14} acid of the same molor specific activity. Decarboxylation by the Schmidt reaction forms nonisotopic putrescine and carbon-C^{14} dioxide; also see aniline-1,2-$C^{14}_{1/2}$. The oxidative step has been effected also with nitric acid.[9]

[1] S. Winstein, T. L. Jacobs, R. M. Henderson and W. H. Florsheim, J. Org. Chem. *11*, 150 (1946).

[2] *Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 185.

[3] A. Kotz, K. Blendermann, R. Rosenbusch, and E. Sirringhaus, Ann., *400*, 55 (1913).

[4] M. Godchot, Compt. rend., *180*, 444 (1925).

[5] *Organic Reactions*, Vol. II, Wiley, New York, 1944, Chapt. 5.

[6]H. Lund, Ber., *70*, 1520 (1937).
[7]P. D. Bartlett, J. Am. Chem. Soc., *57*, 224 (1935).
[8]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 158.
[9]R. B. Loftfield, J. Am. Chem. Soc., *72*, 632 (1950).

ARYL C*-KETONES

METHOD I

$$CH_3C^*OOK \xrightarrow[AlCl_3]{C_6H_6} CH_3C^*OC_6H_5$$

(a)

$$C_6H_5C^*OOH \xrightarrow{SOCl_2} C_6H_5C^*OCl \xrightarrow[AlCl_3]{C_6H_5CH_3} C_6H_5C^*O\text{-}C_6H_4\text{-}CH_3$$

(b)

R. J. Speer and J. K. Jeanes, J. Am. Chem. Soc., *74*, 2443 (1952).

A. Procedure (Note 1)

(a) *Acetophenone-1-C*^{14} (Note 2). A stirred mixture of 10 mmoles of anhydrous potassium acetate-1-C^{14}, 3.91 g. (50 mmoles) of benzene and 5.5 g. (40 mmoles) of anhydrous aluminum chloride is refluxed for 8 hours. The cooled mixture is poured onto cracked ice and acidified with concentrated hydrochloric acid. The aqueous layer is separated and extracted with two 5-ml. portions of benzene. The extract is washed with 2% potassium hydroxide solution and water, to recover unchanged acetic-1-C^{14} acid, then dried over sodium sulfate and evaporated in a dry air stream; crude yield 88% (Note 3). Purification is effected by vacuum distillation (Note 4).

The following analogues are prepared by the same procedure from the appropriate acid:

Propiophenone-1-C^{14}, 78% (Note 5).

Octadecanophenone-1-C^{14}; 80% (Note 6).

4-Chlorobenzophenone-C^{14}, 79% (Note 7).

(b) *4-Methylbenzophenone-C*^{14}. Thionyl chloride (12 mmoles) is added to 10 mmoles of benzoic-C^{14} acid, and the mixture is refluxed for 2 hours. The excess reagent is evaporated under diminished pressure (Note 8), and 50 mmoles of toluene is added, followed by 30 mmoles of anhydrous aluminum chloride in one portion with ice-bath cooling. The mixture is refluxed for 4 hours, then stored for 16 hours at toom temperature and worked up as described above; yield 80% (Note 9).

The following analogues are prepared by the same procedure; the melting points are those of the purified compound:

Benzophenone-C^{14}, 71%, m.p. 47–48°; 2,4-dinitrophenylhydrazone, m.p. 238–239° (Note 10).

4-Methoxybenzophenone-C^{14}, 73%, m.p. 61°; 2,4-dinitrophenylhydrazone, m.p. 180° (Note 11).

1-Acenaphthenone-1-C^{14}, 84%, m.p. 120°; 2,4-dinitrophenylhydrazone, m.p. 230° (Note 12).

1-Indanone-1-C^{14}, 89%, m.p. 42°; 2,4-dinitrophenylhydrazone, m.p. 256° (Note 13).

4′-Bromoacetophenone-1-C^{14}, 47% based on acetyl-1-C^{14} chloride, m.p. 52–53° from petroleum ether.[1]

METHOD II

$$C_6H_5C^*OOH \xrightarrow{SOCl_2} C_6H_5C^*OCl \xrightarrow{(CH_3)_2Cd} C_6H_5C^*OCH_3$$

[1] W. G. Dauben, J. C. Reid, P. E. Yankwich and M. Calvin, J. Am. Chem. Soc., 72, 121 (1950).

A. Procedure

Acetophenone-1-C^{14}. From 52.5 g. (374 mmoles) of benzoyl-C^{14} chloride and dimethylcadmium is obtained 38.0 g. (b.p. 84–85° at 20 mm.) of acetophenone-1-C^{14}, in 85% yield, by suitable adaptation of the method of Gilman and Nelson[1a] as modified by Cason,[2] which follows (for the preparation of propiophenone).

A Grignard reagent is prepared from 0.3 mole of ethyl bromide (Note 14) and magnesium in absolute ether under nitrogen. To the ice-cold solution is added 28.6 g. (0.156 mole) of powdered anhydrous cadmium chloride during 7 minutes; then the mixture is stirred under reflux until a negative Grignard test[1a,3] is obtained (20 minutes). The ether is distilled on a steam-bath until the residue is nearly dry; then 65 ml. of dry benzene is added, and 25 ml. of additional distillate is collected. To the stirred solution, diluted to 170 ml. with benzene and cooled to 10°, is added 0.21 mole of benzoyl chloride in 50 ml. of benzene during 2 minutes (Note 15). The mixture is stirred for 1.5 hours at 40–45°, the reaction remaining exothermic for about 40 minutes. The organometallic complex is decomposed with ice and sulfuric acid; then the aqueous phase is separated and extracted with benzene. The benzene solution is washed with water, 5% sodium carbonate solution, water and saturated sodium chloride solution, then filtered slowly through anhydrous sodium sulfate. The solvent is flashed off, and the residue is distilled through a Podbielniak column. The yield of propiophenone is 84.4% based on acid chloride (Note 16).

Acetophenone-1-C^{14} has been prepared in 24% yield by Roberts,[4] by reacting acetyl-1-C^{14} chloride with diphenylcadmium.

METHOD III

$$C_6H_5C^*OOH \xrightarrow{SOCl_2} C_6H_5C^*OCl \xrightarrow[Na]{CH_2(COOC_2H_5)_2}$$

$$C_6H_5C^*OCH(COOC_2H_5)_2 \xrightarrow[(HCl)]{KOH} C_6H_5C^*OCH(COOH)_2 \xrightarrow[\Delta]{HCl} C_6H_5C^*OCH_3$$

W. G. Dauben, J. C. Reid, P. E. Yankwich and M. Calvin, J. Am. Chem. Soc., 72, 121 (1950).

A. Procedure

Acetophenone-1-C^{14}. From 2.81 g. (20 mmoles) of benzoyl-C^{14} chloride and 16 g. (100 mmoles) of ethyl malonate is prepared 1.89 g. of acetophenone-1-C^{14} in 78.5% yield, by a suitable adaptation of the method of Wilds and Beck,[5] which follows (for the preparation of 2-acetonyl-3,4-dihydro-2-methyl-1(2*H*)-phenanthrone). Also see 1-phenyl-2-propanone-1-C^{14}, Note 2.

A benzene solution (250 ml.) of the acid chloride (prepared from 89.5 mmoles of 1,2,3,4-tetrahydro-2-methyl-1-oxo-2-phenanthreneacetic acid) is added to a cooled suspension of sodiummalonic ester, prepared by refluxing for 7 hours a mixture of 4.2 g. of powdered sodium and 43 ml. of ethyl malonate in 500 ml. of absolute ether. The mixture is stirred for 12 hours at room temperature and refluxed for 2 hours, then diluted with water, acidified with acetic acid and extracted with ether. The extract is concentrated, and the residue is hydrolyzed and decarboxylated by refluxing for 4 hours with 250 ml. of concentrated hydrochloric acid, 250 ml. of acetic acid and 100 ml. of water. The mixture is diluted with water, and the product is extracted into benzene solution, which is washed with water and sodium bicarbonate solution and evaporated to dryness. The crude 2-acetonyl-3,4-dihydro-2-methyl-1(2*H*)-phenanthrone is recrystallized from methanol; yield 83%, m.p. 106–109°.

METHOD IV

$$CH_3C^*N \xrightarrow{C_6H_5MgBr} CH_3C^*(C_6H_5)=NMgBr \longrightarrow CH_3C^*(C_6H_5)=NH\cdot HCl \xrightarrow{H_2O} CH_3C^*OC_6H_5$$

E. V. Brown, E. Cerwonka and R. C. Anderson, J. Am. Chem. Soc., 73, 3735 (1951).

A. Procedure

Acetophenone-1-C^{14} (Note 17). A dried benzene solution of acetonitrile-1-C^{14}, prepared from sodium cyanide-C^{14} and methyl sulfate (100 mmoles each), is added slowly to a stirred solution of 4 molar amounts of phenylmagnesium bromide in ether. The mixture is stirred for 1 hour, stored overnight at room temperature, then poured into a mixture of ice and hydrochloric acid. The aqueous layer is separated, heated under reflux for 1 hour to hydrolyze the α-methylbenzylidenimine-C^{14} hydrochloride, then extracted with four portions of ether. The extract is dried over calcium chloride and concentrated on a water-bath, and the residue is vacuum-distilled; yield 7.5 g. (70%), b.p. 83–85° (12 mm.), 63% based on sodium cyanide.

Acetophenone-2-C^{14} is prepared from acetonitrile-2-C^{14} by the same procedure; yield 53% based on methyl-C^{14} iodide.

METHOD V

$$(CH_3C^*O)_2O \xrightarrow[AlCl_3]{C_6H_5Cl} Cl\text{-}C_6H_4\text{-}C^*OCH_3$$

R. Macdonald and B. E. Baker, Can. J. Chem., *31*, 517 (1953).

A. Procedure

4′-Chloroacetophenone-1-C^{14}. A mixture of bis(acetic-1-C^{14}) anhydride (prepared from 7.4 mmoles of sodium acetate-1-C^{14}), 2.4 g. of anhydrous aluminum chloride and 1.40 g. of chlorobenzene is refluxed at 100° for 18 hours. The mixture is cooled to 50–60° and treated with 4 g. of ice and 4 ml. of concentrated sulfuric acid. The product is steam-distilled from the mixture and isolated by ether extraction and vacuum distillation. The radiochemical yield is 61% based on bis(acetic-1-C^{14}) anhydride.

Acetophenone-1-C^{13} is prepared[6] similarly by condensing bis(acetic-1-C^{13}) anhydride with benzene; yield 43% based on sodium acetate-1-C^{13}.

METHOD VI

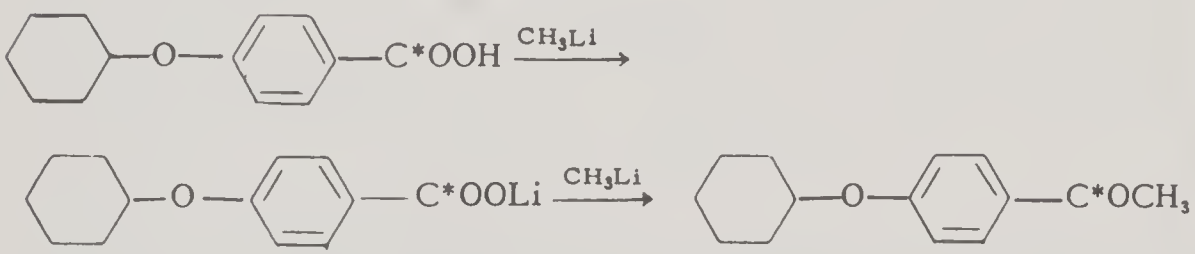
$$C^*H_3COOH \xrightarrow{PCl_5} C^*H_3COCl \xrightarrow[AlCl_3]{C_6H_5} C^*H_3COC_6H_5$$

C. H. Shuford, Jr., D. L. West and H. W. Davis, J. Am. Chem. Soc., 76, 5803 (1954); H. W. Davis, Atomic Energy Commission Report, ORO-122; Nuc. Sci. Abstracts, 8, 4025 (1954).

A. Procedure (Note 18)

Acetophenone-2-C^{14}. A mixture of 12.0 g. of glacial acetic acid and 0.085 g. of sodium acetate-2-C^{14}, equilibrated for 3 hours, is added dropwise to a stirred, cooled mixture of 46 g. (0.22 mole) of phosphorus pentachloride and 60 g. (0.77 mole) of benzene (Note 19). This is followed by 80 g. (0.60 mole) of anhydrous aluminum chloride over a period of 30 minutes. After washing of the reflux condenser with 10 ml. of benzene, the mixture is refluxed for 30 minutes, cooled, poured into 100 ml. of concentrated hydrochloric acid and 900 g. of ice, and stored overnight. The benzene layer is separated, washed with two 100-ml. portions of 10% sodium hydroxide solution, and dried, and the solvent is distilled off through a short column. The product is collected by distillation through a 3-foot Vigreux column; yield 18.0 g. (75%), b.p. 90–91° (20 mm.).

METHOD VII



J. B. Brierley and A. McCoubrey, Brit. J. Pharmacol., *8*, 366 (1953).

A. Procedure (Note 20)

4′-Cyclohexyloxyacetophenone-1-C^{14}. To a dry ethereal solution of *p*-cyclohexyloxybenzoic-C^{14} acid is added slowly three molecular proportions of methyllithium, and the mixture is refluxed for 16 hours. The product melts at 79° and boils at 145° (0.6 mm.); oxime, m.p. 122°.

From *p*-(1-methylethoxy)benzoic-C^{14} acid is prepared 4′-(1-methylethoxy)acetophenone-1-C^{14}, m.p. 22°, b.p. 115° (bath temperature) (13 mm.); oxime, m.p. 112°.

B. Notes

1. The method is general for the synthesis of functionally labeled alkyl-aryl, diaryl and alicyclic ketones from the carboxylic acids.

2. The procedure is a modification of the method of Shantz and Rittenberg;[6] see Method V.

3. The preparation, employing acetic-1-C^{14} acid, has been reported in 78.8% yield (b.p. 195–199°) by Neville,[7] and 83% yield by Roberts,[8] while Ropp[9] employed acetyl-1-C^{14} chloride.

4. The product is assayed as the 2,4-dinitrophenylhydrazone.[10] Ropp[9] has reported an isotope effect in the formation of this derivative and the semicarbazone. The effect is due to rate and not exchange.

The heterogeneous catalytic reduction of acetophenone-1-C^{14} to α-methylbenzyl-α-C^{14} alcohol has been described by Bonner.[11] An 11.5% isotope effect is reported with Adams catalyst in ethanol at room temperature and 1 atmosphere. Experiment showed that there was no exchange between product and starting material. An 8% isotope effect was observed when reduction was accomplished with lithium aluminum hydride in ether. The carbinol was assayed as the 3,5-dinitrobenzoate ester, m.p. 99–100°, after two recrystallizations from alcohol.

5. Roberts[12] has prepared propiophenone-2-C^{14} from sodium propionate-2-C^{14} by this procedure.

6. Purification by repeated recrystallization from alcohol gives a product melting at 58–60°; 2,4-dinitrophenylhydrazone, m.p. 91–93°. The free acid is employed as starting material.

7. The starting material is benzoic-C^{14} acid.

8. In the case of less volatile acid chlorides, the last traces of thionyl chloride are removed by addition of dry benzene and re-evaporation under reduced pressure.

9. Purification is effected by recrystallization from dilute alcohol, m.p. 59–60°; 2,4-dinitrophenylhydrazone, m.p. 198°.

10. A yield of 91% at the 25-mmole level has been reported by Geissman.[13] Benzophenone-C^{14} has been prepared in a similar manner by Fleming (see C_1^{14}-benadryl, Method II), employing the acid chloride and carbon bisulfide solvent (yield 88%). The preparation in 72% yield (38% radiochemical yield) from a mixture of benzoyl chloride and benzoic-C^{14} acid in carbon disulfide has been described by Burr.[14] An isotope effect in the formation of the 2,4-dinitrophenylhydrazone has been reported by Ropp[9] and Brown.[15] The oxime melts at 141°. The preparation of benzophenone-C^{14} by essentially the same procedure has been described by Brown.[15]

11. Equimolar amounts of the acid chloride and anisole, in cold carbon disulfide (25 ml.), are treated with aluminum chloride and stored overnight at room temperature, before heating under reflux for 30 minutes.

12. 1-Naphthaleneacetyl-C^{14} chloride, in ice-cold isohexane (25 ml.), is treated with aluminum chloride. After 1 hour at 0°, the mixture is warmed to solution (50°) and stored overnight at room temperature.

13. The reaction conditions are those described for 1-acenaphthenone-1-C^{14}.

14. In the preparation of ketones by the dialkylcadmium method, iodides give much lower yields than do bromides, while chlorides are somewhat inferior to the latter.

15. Ice-bath cooling is employed to prevent the temperature from rising above 15°.

16. The yield was 70.5% when the acid chloride solution was added to the boiling dialkylcadmium solution, and refluxing was continued for 1 hour. A lower reaction temperature appears to be desirable in those cases where there is no difficulty with a stirrer-stopping precipitate.

17. The procedure is adapted from the method of Shriner[16] for preparing alkyl phenyl ketones from nitriles; acetophenone (70%), b.p. 202–205°, n_D^{20} 1.541.

18. The method is essentially that of Ullmann.[17]

19. The rate of addition is determined by the rate of evolution of hydrogen chloride.

20. The method is that of Gilman[18] for converting acids to the corresponding ketones by reaction of the lithium salt with organolithium. Also see 4-phenyl-3-buten-2-one-2-C^{13}, 6-hydroxy-6-methyl-2-heptanone-1-C^{14}, C^{14}-vitamin A_2 Note 2, and McCoubrey.[19]

C. Other Preparations

4′-Hydroxypropiophenone-1-C^{14} has been prepared[20] by condensing propionic-1-C^{14} acid with phenol in the presence of boron trifluoride.

[1]A. A. Bothner-By, J. Am. Chem. Soc., 77, 3293 (1955); *Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 109.

[1a]H. Gilman and J. F. Nelson, Rec. trav. chim., 55, 518 (1936).

[2]J. Cason, J. Am. Chem. Soc., 68, 2078 (1946).

[3]H. Gilman and F. Schulze, J. Am. Chem. Soc., 47, 2002 (1925).

[4]J. D. Roberts, Nucleonics, 7 (4), 45 (1950).

[5]A. L. Wilds and L. W. Beck, J. Am. Chem. Soc., 66, 1688 (1944); M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 200.

[6]E. M. Shantz and D. Rittenberg, J. Am. Chem. Soc., 68, 2109 (1946).

[7]O. K. Neville, J. Am. Chem. Soc., 70, 3499 (1948); M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 201.

[8]J. D. Roberts, D. R. Smith and C. C. Lee, J. Am. Chem. Soc., 73, 618 (1951).

[9]G. A. Ropp, V. F. Raaen and A. J. Weinberger, J. Am. Chem. Soc., 75, 3694 (1953).

[10]H. A. Iddles, A. W. Low, B. D. Rosen and R. T. Hart, Ind. Eng. Chem., Anal. Ed., *11*, 102 (1939).

[11]W. A. Bonner and C. J. Collins, J. Am. Chem. Soc., 75, 4516 (1953).

[12]R. M. Roberts and S. G. Brandenberger, Chemistry and Industry, *1955*, 227.

[13]T. A. Geissman, Atomic Energy Commission Report, UCRL-1233; Nuc. Sci. Abstracts, 5, 4722 (1951).

[14]J. G. Burr, Jr., J. Am. Chem. Soc., 75, 1990 (1953).

[15]F. Brown and D. Holland, Can. J. Chem., 30, 438 (1952).

[16]R. L. Shriner and T. A. Turner, J. Am. Chem. Soc., 52, 1267 (1930).

[17]F. Ullmann and H. Bleier, Ber., 35, 4273 (1902); *Organic Syntheses*, Vol. 32, Wiley, New York, 1952, p. 8.

[18]H. Gilman and P. R. Van Ess, J. Am. Chem. Soc., *55*, 1258 (1933).
[19]A. McCoubrey and N. K. Iyengar, J. Chem. Soc., *1951*, 3430.
[20]P. Daudel, *et al.*, Congr. Intern. biochim., Résumés communs., 2e Congr., Paris, *1952*, 466.

2-PHENYLACETOPHENONE-1-C^{14}
(C_1^{14}-Deoxybenzoin)

$$C_6H_5C^*OCH_3 \xrightarrow[NaOH]{C_6H_5CHO} C_6H_5C^*OCH{=}CHC_6H_5 \xrightarrow{H_2O_2}$$

(a)

$$C_6H_5C^*O\underset{\diagdown O \diagup}{CHCH}C_6H_5 \xrightarrow{NaOH} C_6H_5C^*OCOCH_2C_6H_5 \xrightarrow{NaOH}$$

(b)

$$C_6H_5\overset{\displaystyle COOH}{\underset{\displaystyle OH}{C^*}}CH_2C_6H_5 \xrightarrow{CrO_3} C_6H_5C^*OCH_2C_6H_5 \xrightarrow{NH_2OH} C_6H_5\overset{\displaystyle NOH}{\underset{}{C^*}}CH_2C_6H_5 \xrightarrow{PCl_5}$$

(c) (d)

$$C_6H_5NHC^*OCH_2C_6H_5$$

(e)

C. J. Collins and O. K. Neville, J. Am. Chem. Soc., *73*, 2471 (1951).

A. Procedure

(a) *Chalcone-C^{14}*, (*2-Benzylideneacetophenone-1-C^{14}*) (Note 1). Acetophenone-1-C^{14} (1.4 g.) is condensed with 1.4 g. of benzaldehyde in alcoholic sodium hydroxide solution, by scaling down the procedure of Kohler and Chadwell.[1] The yield of crude product is 2.35 g. (93%), and one recrystallization from alcohol gives material melting at 51–58°.

(b) *2,3-Epoxy-3-phenylpropiophenone-1-C^{14}*, (*2-Benzylideneacetophenone-1-C^{14} Oxide*). 2-Benzylidineacetophenone-1-C^{14} (1.96 g.) is converted to the epoxide (m.p. 86.5–87.5°) in 2.01-g. yield (95%), by the method of Weitz and Scheffer,[2] which follows.

A cooled solution of 2.08 g. (10 mmoles) of 2-benzylidineacetophenone in 25 ml. of methanol is mixed with 3 ml. of 15% hydrogen peroxide (12 mmoles) and 2.5 ml. of 2 *N* sodium hydroxide, the temperature being kept below 30°. The crystalline mass is collected and washed with a little methanol. The yield is 1.8 g., and dilution of the mother liquor with water gives 0.2 g. of additional product. Recrystallized from 4–5 parts of

methanol, the compound melts at 90°, and further heating produces a light explosion.

(c) *α-Benzylmandelic-α-C¹⁴ Acid* (Note 2). A mixture of 1.52 g. of 2-benzylideneacetophenone-1-C¹⁴ oxide, 15 ml. of ethanol and 3 ml. of 6 *N* sodium hydroxide is refluxed for 1.5 hours, then diluted with 60 ml. of water and saturated with carbon dioxide. The solution is washed with ether and acidified with dilute hydrochloric acid to precipitate the product; yield 1.14 g., 72%. Recrystallization from benzene-petroleum ether gives colorless product, m.p. 165–166°.

(d) *2-Phenylacetophenone-1-C¹⁴*. To a solution of 0.4635 g. of the hydroxy acid in 30 ml. of glacial acetic is added 0.080 g. of chromic anhydride. Nitrogen is bubbled slowly through the stirred solution for 30 minutes at room temperature (Note 3); then the mixture is diluted, by being poured into 150 ml. of water, and continuously extracted with ether for 4 hours. The extract is washed with sodium bicarbonate solution, dried and concentrated; yield 0.342 g. (91%). Colorless crystals are produced by two crystallizations from alcohol, m.p. 51.0–52.5°. The molar specific activity is the same as that of the starting material (Note 4).

(e) *2-Phenylacetanilide-1-C¹⁴*. 2-Phenylacetophenone-1-C¹⁴ (0.5 g.) is converted to 0.528 g. of crude oxime (m.p. 85–93°) by treatment with 0.5 g. of hydroxylamine hydrochloride. A mixture of the oxime, 5 ml. of benzene and 0.311 g. of phosphorus pentachloride is refluxed for 15 minutes, then diluted with 20 ml. of water, and the benzene is removed in a stream of air. The crude product is extracted into ether solution, which is washed with bicarbonate solution and water and evaporated; yield 0.481 g., m.p. 105–113°. The crude product, in 4.0 ml. of benzene, is placed on an alumina column, which is developed with 30 ml. of benzene (Note 5). Elution of the column with ethanol, followed by evaporation, yields 0.379 g. (79%) of crude product. Decolorization with charcoal and recrystallization from ether-petroleum ether give 0.328 g. of colorless crystals (68%), m.p. 114–115°. Degradation studies (Note 6) indicate that the isotope occurs only at C-1.

B. Notes

1. Roberts[3] has reported the preparation (100% yield) by employing sodium ethoxide as the condensing agent. The dibromide was prepared by the addition of bromine (77% yield).

2. Widman[4] has reported a yield of 7 g. (m.p. 165–166°) from 10 g. of starting material, by this rearrangement. The method is of value in preparing some unsymmetrical deoxybenzoins and glycolic acids.[5] The intermediate benzyl phenyl diketone may be isolated after short treatment

of the epoxide with alcoholic alkali. For a similar alkaline rearrangement of an α-dicarbonyl compound, see mandelic-α-C^{14} acid.

3. The evolved carbon dioxide is found to have very little activity.

4. This degradation rules out the possibility that the alkaline rearrangement of benzyl phenyl diketone (the α-diketone intermediate arising from the epoxide, b) might take place by phenyl, rather than by benzyl, group migration (see Malkin).[6]

5. The column is 11 × 140 mm., 80–200 mesh. The first 14 ml. of benzene eluate contains a viscous oil.

6. Directions are given by Collins and Neville for hydrolyzing the anilide quantitatively to phenylacetic-1-C^{14} acid, by heating in a sealed tube with acetic-hydrochloric acid mixture. The molar specific activity is the same as that of the starting materials. Oxidation with chromic acid and permanganate degrades this acid to nonisotopic benzoic acid. This data rules out any possibility of isomerism of the epoxide (b).

C. Other Preparations

Hendley[7] and Clark[8] have prepared a similar series of compounds from *p*-anisaldehyde by the described procedures, with the following yields and melting points:

4-Methoxychalcone-C^{14}, 87%, m.p. 70°.

2,3-Epoxy-3-(*p*-methoxyphenyl)propiophenone-1-C^{14}, 50%, m.p. 82–83°.

α-(*p*-Methoxybenzyl)mandelic-α-C^{14} acid, 44%, m.p. 192.5–193.0°.

2-(*p*-Methoxyphenyl)acetophenone-1-C^{14}, 86%, m.p. 96°; over-all radiochemical yield 32.3% based on acetophenone.

Degradation studies indicate that the isotope distribution still follows the pattern of the first series, with exclusive benzyl (substituted) group migration. Although the *p*-methoxyphenyl group has been shown by Roberts[9] to migrate to a much less extent than the phenyl group in the benzilic acid rearrangement of *p*-methoxybenzil (q.v.), the presence of a *p*-methoxyl group on the ring of the potential benzyl group in the present case does not promote phenyl group migration. The yield of by-product barium carbonate is 96%.

From benzaldehyde and 4′-methoxyacetophenone-1-C^{14}, Roberts[9] has prepared 4′-methoxychalcone-C^{14} in 87% yield, m.p. 103–104.5°.

[1] *Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 78.
[2] E. Weitz and A. Scheffer, Ber., *54*, 2327 (1921).
[3] J. D. Roberts, Nucleonics, 7 (4), 45 (1950).
[4] O. Widman, Ber., *49*, 477 (1916).
[5] W. Baker and R. Robinson, J. Chem. Soc., *1932*, 1798; W. A. Hutchins, D. C. Motwani, K. D. Mudbhatkal and T. S. Wheeler, J. Chem. Soc., *1938*, 1882.
[6] T. Malkin and R. J. Robinson, J. Chem. Soc., *1925*, 371.
[7] E. C. Hendley and O. K. Neville, J. Am. Chem. Soc., *75*, 1995 (1953).

[8]M. T. Clark, E. G. Hendley and O. K. Neville, J. Am. Chem. Soc., 77, 3280 (1955).

[9]J. D. Roberts, D. R. Smith and C. C. Lee, J. Am. Chem. Soc., 73, 618 (1951).

3-DIMETHYLAMINOPROPIOPHENONE-3-C^{14} HYDROCHLORIDE

$$C_6H_5COCH_3 + C^*H_2O + NH(CH_3)_2 \cdot HCl \xrightarrow[C_2H_5OH]{}$$

$$C_6H_5COCH_2C^*H_2N(CH_3)_2 \cdot HCl \xrightarrow[\text{(NaOH)}]{H_2O,\ \triangle} C_6H_5COCH{=}C^*H_2 + (CH_3)_2NH$$

$$C_6H_5COCH{=}C^*H_2 \xrightarrow{O_3} C_6H_5COCHO + C^*H_2O$$

A. V. Logan, J. L. Huston and D. L. Dorward, J. Am. Chem. Soc., 73, 2952 (1951).

A. Procedure (Note 1)

A mixture of 1.84 g. (60 mmoles) of paraformaldehyde-C^{14}, 9.70 g. (120 mmoles) of dimethylamine hydrochloride, 10 ml. of acetophenone and 10 ml. of 95% ethanol is refluxed for 2 hours, then chilled overnight. The solid is collected and recrystallized from acetone; yield 6.62 g. (51%), mp. 155–157°. Degradation studies indicate that there is no isotopic rearrangement (Note 2) during formation of the Mannich base.

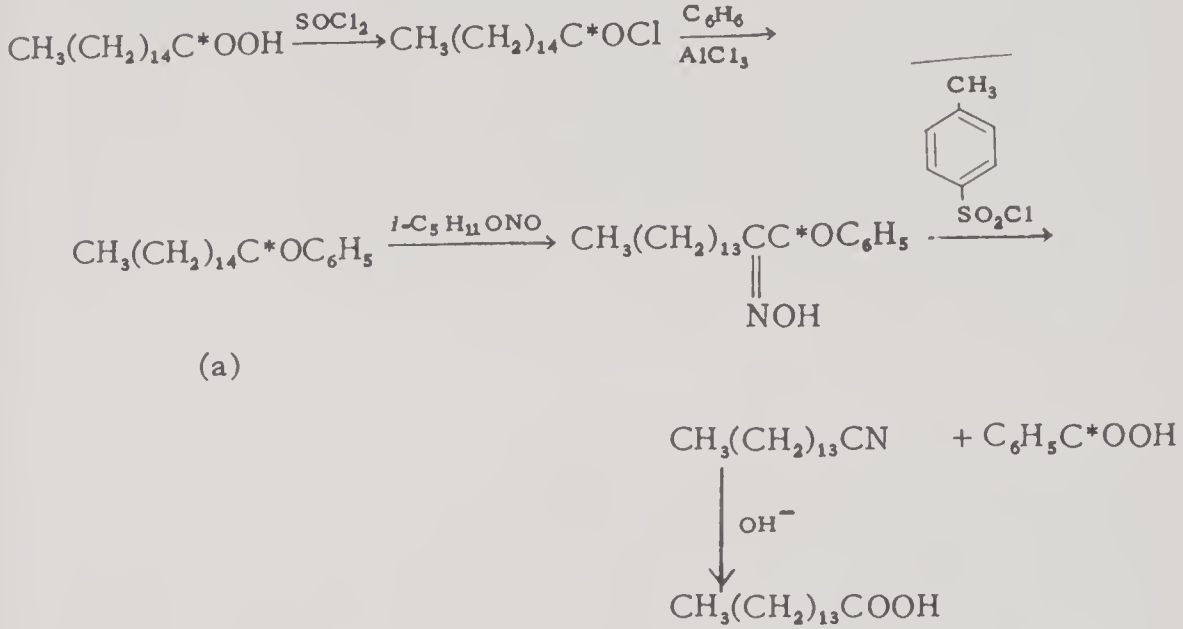
B. Notes

1. The reaction conditions are modified from those normally used for preparing the β-keto amine of the type $RCOCH_2CH_2NR'_2$ by the Mannich reaction.[1]

2. The product was pyrolyzed by steam distillation, and the acrylophenone-3-C^{14} formed was extracted from the distillate with chloroform. The chloroform was distilled, and the residue was ozonized in glacial acetic acid solution at 80° for 4 hours. The mixture then was poured into water containing zinc to prevent oxidation, and the precipitate of phenylglyoxal monohydrate was collected and converted to the dimedon derivative; yield 0.320 g. m.p. 182–185°. The filtered solution of formaldehyde-C^{14} was converted to the dimedon derivative for assay.

[1]J. T. Plati and W. Wenner, J. Org. Chem., *14*, 543 (1949); F. F. Blicke and J. H. Burckhalter, J. Am. Chem. Soc., *64*, 451 (1942); *Organic Reactions*, Vol. I, Wiley, New York, 1942, Chapter 10; C. Mannich and G. Heilner, Ber., *55*, 356 (1922).

HEXADECANOPHENONE-1-C^{14}
(C_1^{14}-Palmitophenone)



W. G. Dauben, E. Hoerger and J. W. Petersen, J. Am. Chem. Soc., 75, 2347 (1953).

A. Procedure (Note 1)

(a) *Hexadecanophenone-1-C^{14}*. A stirred mixture of 0.823 g. (3.2 mmoles) of hexadecanoic-1-C^{14} acid, 1.5 ml. (20 mmoles) of purified thionyl chloride and 10 μl. of pyridine is heated at 40° for 30 minutes. The excess thionyl chloride is removed under reduced pressure, and the residual hexadecanoyl-1-C^{14} chloride is dissolved in 3.5 ml. (30 mmoles) of anhydrous benzene. The solution is cooled to 0°, 0.750 g. (5.6 mmoles) of anhydrous aluminum chloride is added, and the mixture is stirred for 12–16 hours at room temperature. Dilute hydrochloric acid is added, and product is extracted into hexane, which is separated, washed with 5 ml. of *N* sodium hydroxide and 1:2 methanol-water, then evaporated to dryness. The residue is twice recrystallized from hexane; yield 0.908 g. (89%), m.p. 58–59°.

Benzoic-C^{14} Acid (Note 2). To a stirred mixture at 50° of 0.908 g. (2.86 mmoles) of the palmitophenone, 10 ml. of purified dioxane and 0.6 ml. of concentrated hydrochloric acid is slowly added, over a period of 45 minutes, a solution of 0.49 ml. (3.9 mmoles) of freshly distilled isopentyl nitrite and 5 ml. of dioxane (Note 3). After another 15 minutes at 50°, the solution is made basic by the addition of 12 ml. (36 mmoles) of

3 *N* sodium hydroxide. The mixture is cooled to room temperature and, without isolating the 2-hydroxyiminohexadecanophenone-1-C^{14} (Note 4), 2.0 g. (10 mmoles) of *p*-toluenesulfonyl chloride is added slowly with stirring over 20 minutes. The mixture is stirred at 50° for 2 hours, then diluted with 300 ml. of water and extracted with pentane. The cooled aqueous solution is filtered (Note 5), and the filtrate is acidified with hydrochloric acid and extracted with several small portions of ether (Note 6). The ether solutions are evaporated in a sublimator, and the residue is sublimed at 80° at 200 mm., with the first few per cent of sublimate being discarded; yield 0.278 g. (79%), m.p. 121–122°.

B. Notes

1. The procedure is adapted from a method of Darzens and Mentzer,[1] and has been developed as a method for degrading acids, one carbon at a time, in such a manner that the process may be continuously repeated. The yield of the next lower acid is 50–75%, and no extraneous carbon is introduced in the process. The degradation carbon appears as the carboxyl of benzoic acid, which is convenient for isolating in excellent yield on a small scale. Other methods for degrading fatty acids have been discussed by Dauben.

2. The method involves a second-order Beckmann rearrangement of an α-diketone monoxime, α-isonitrosoketone, in the presence of *p*-toluenesulfonyl chloride and alkali.[1,2]

3. A convenient dropping funnel is described by Hershberg.[3]

4. A number of α-hydroxyimino ketones have been prepared by a similar procedure.[4]

5. The sodium pentadecanoate collected yields 30 mg. of acid.

6. The pentane extract is evaporated to dryness, and the residue of pentadecanenitrile is hydrolyzed by refluxing with 10 ml. of 15% potassium hydroxide in 1-propanol for 24 hours. The mixture is diluted with hexane, and the copious precipitate of sodium pentadecanoate is filtered off. The filtrate is evaporated to dryness, and the residue is washed with hexane and combined with the soap already collected. The salt is acidified, and the acid is extracted into pentane. The solvent is evaporated, and the crude pentadecanoic acid is twice recrystallized from pentane; yield 0.485 g. (70%), m.p. 51–52°.

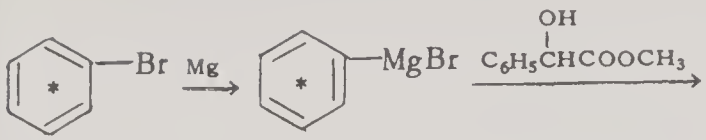
[1] G. Darzens and C. Mentzer, Compt. rend., *213*, 268 (1941).

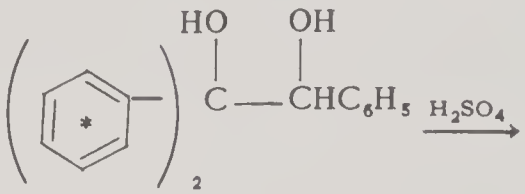
[2] L. Claisen and O. Manasse, Ber., *20*, 2194 (1887).

[3] *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 129.

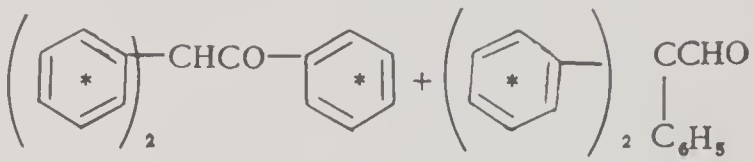
[4] W. H. Hartung, J. C. Munch, W. A. Deckert and F. Crossley, J. Am. Chem. Soc., *52*, 3317 (1930).

$C^{14}_{2/26}$-2,2-DIPHENYLACETOPHENONE





(a)



(b)

C. J. Collins, J. Am. Chem. Soc., 77, 5517 (1955); C. J. Collins and G. A. Ropp, J. Am. Chem. Soc., 77, 4160 (1955).

A. Procedure (Note 1)

(a) *α-(α-Hydroxybenzyl)benzhydrol-(1,1′), (1,2′), (1,3′), (1,4′), (2,2′), (2,3′), (2,4′), (3,3′), (3,4′), (4,4′)-*$C^{14}_{2/10}$, [*1,1-Bis(phenyl-*$C^{14}_{1/4}$*)-2-phenyl-1,2-ethanediol*]. Methyl mandelate is condensed with phenylmagnesium-$C^{14}_{1/4}$ bromide according to the procedure of Acree,[1] which follows. The product melts at 165–167°, and the molar specific activity is twice that of the starting material (Note 2).

To an ethereal Grignard solution prepared from 11 g. of bromobenzene is added 3 g. of methyl mandelate in ether. The mixture is refluxed for 2 hours and worked up; yield 4.0 g., m.p. 167° from alcohol.

(b) $C^{14}_{2/26}$-*2,2-Diphenylacetophenone* (Note 3). To a quantity of 0.500 g. of the glycol is added 10 ml. of cold concentrated sulfuric acid (Note 4), and the mixture is stirred for 5 minutes, stored in a refrigerator for 4 hours and poured over 25–50 g. of crushed ice. The white precipitate is extracted with four portions of ether, and the extract is washed with water and bicarbonate solution and evaporated. The residue is crystallized from 30–40 ml. of alcohol (Norit); yield 0.300–0.375 g.

(64–80%), m.p. 135.5–136.5° (Note 5). Degradation studies (Note 6) indicate that 70.2% of the activity is located in the 2-phenyl rings.

B. Notes

1. These preparations are incidental to a determination of the relative importances of four reaction mechanisms that have been proposed[2] for the pinacol rearrangement of triphenylethylene glycol[3] under the influence of five different acidic catalysts.

2. Proof of structure was established by oxidizing the glycol with permanganate (see triphenylethylene-$C^{14}_{1/2}$, Note 6) forming benzoic acid and benzophenone-(1,1′),(1,2′),(1,3′),(1,4′),(2,2′),(2,3′),(2,4′),(3,3′),(3,4′),(4,4′)-$C^{14}_{2/10}$ having the same molar specific activity.

3. For lack of sufficient parameters the system of nomenclature cannot name this product, which is a mixture of 26 structures: 2-phenyl-k-C^{14}-2-phenyl-m-C^{14}-acetophenone-n-C^{14}, where k, m and n have values 0 to 4, k and m not being zero at the same time.

4. The acidic catalysts: cold concentrated sulfuric acid, refluxing formic acid (17 hours), refluxing oxalic acid dihydrate (8 g., 8 hours), 12 *N* sulfuric acid (6 hours) and refluxing dioxane-concentrated hydrochloric acid (16.6 ml.:25 ml., 8 hours) give, respectively, the following per cent C^{14}-migration: 29.8, 21.1, 19.6, 7.0 and 0. The rearrangement product in the latter case (unrearranged isotopically) is benzhydryl-(1,1′),(1,2′),(1,3′),(1,4′),(2,2′),(2,3′),(2,4′),(3,3′),(3,4′),(4,4′)-$C^{14}_{2/10}$ phenyl ketone, ($C^{14}_{2/10}$-2,2-diphenylacetophenone).

5. Isotopic dilution[4] studies indicate that the yield is quantitative, only 0.77% of α-phenylbenzhydryl-(1,1′),(1,2′),(1,3′),(1,4′),(2,2′),(2,3′),(2,4′),(3,3′),(3,4′),(4,4′)-$C^{14}_{2/10}$-formaldehyde, [bis(phenyl-$C^{14}_{1/4}$)phenylacetaldehyde], being formed. Similar studies indicate that the formation of the aldehyde is 29.9, 16.5, 3.9 and 0.98%, respectively, in the presence of oxalic acid dihydrate, dilute sulfuric acid, dioxane-hydrochloric acid and formic acid.

6. The product is not rearranged by dissolution in concentrated sulfuric acid during 24 hours. Permanganate oxidation forms $C^{14}_{2/10}$-benzophenone having a molar specific activity 70.2% of that of the starting material.

C. Other Preparations

1,1,2-Triphenyl-1,2-ethanediol-1-C^{14} is prepared from methyl mandelate-C^{14} by the same procedure.

In a similar manner[5] has been prepared 1-phenyl-$C^{14}_{1/4}$-1,2-diphenyl-1,2-ethanediol (m.p. 165–167°) by condensation of phenylmagnesium-$C^{14}_{1/4}$

bromide and benzoin.[1,6] The molar specific activity of this isotopic isomer of compound (a) was the same as that of the starting material.

α-Phenylbenzoin-1′,2′,3′,4′-$C^{14}_{1/4}$ has been prepared by condensation of phenylmagnesium-$C^{14}_{1/4}$ bromide with benzil.

By the same rearrangement procedure from 1,1,2-triphenyl-1,2-ethanediol-2-C^{14} have been prepared 2,2-diphenylacetophenone-1,2-$C^{14}_{1/2}$ and triphenylacetaldehyde-1-C^{14}.

2,2,2-Triphenylacetophenone-1,2-$C^{14}_{1/2}$ has been prepared[7] by rearrangement of tetraphenyl-1,2-ethanediol-1-C^{14}, (C^{14}_1-benzopinacol). The degradation products, benzoic-C^{14} acid and triphenylmethane-C^{14}, were found to have the same molar specific activity, indicating the absence of any intramolecular isotope effect in the rearrangement and the improbability of some of the postulated detailed reaction mechanisms.

[1]S. F. Acree, Ber., *37*, 2753 (1904).

[2]A. McKenzie, R. Roger and W. B. McKay, J. Chem. Soc., *1932*, 2597; A. McKenzie, A. K. Mills and J. R. Myles, Ber., *63*, 904 (1930); R. Roger and W. B. McKay, J. Chem. Soc., *1933*, 332; G. W. Wheland, *Advanced Organic Chemistry*, Wiley, New York, 1949, Chapter 12.

[3]M. Tiffeneau, Compt. rend., *146*, 29 (1908); R. Lagrave, Ann. Chim., (10) *8*, 416 (1927); S. Danilov and E. V. Danilova, Ber., *59*, 377 (1926).

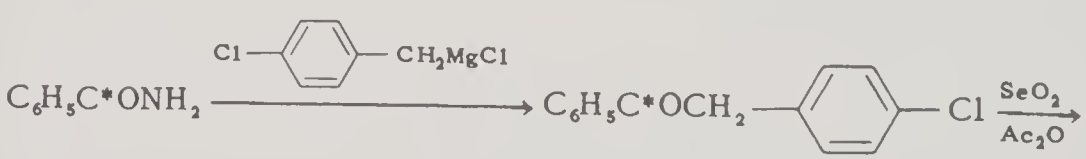
[4]R. H. Mayor and C. J. Collins, J. Am. Chem. Soc., *73*, 471 (1951).

[5]K. Mislow and M. Siegel, J. Am. Chem. Soc., *74*, 1060 (1952).

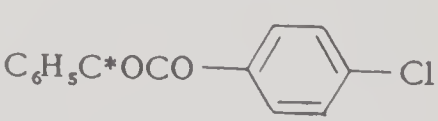
[6]A. McKenzie and H. Wren, J. Chem. Soc., *97*, 473 (1910).

[7]W. Carrick and A. Fry, Anal. Chem., *25*, 1933 (1953).

1-(*p*-CHLOROPHENYL)-2-PHENYLGLYOXAL-2-C^{14}



(a)



(b)

M. T. Clark, E. C. Hendley and O. K. Neville, J. Am. Chem. Soc., 77, 3280 (1955).

A. Procedure

(a) *2-(p-Chlorophenyl)acetophenone-1-C*^{14} (Note 1). In a 100-ml. three-necked flask fitted with a stirrer, nitrogen inlet and reflux condenser is prepared a Grignard solution from 16.0 g. of *p*-chlorobenzyl chloride and 2.4 g. of magnesium in 50 ml. of absolute ether (Note 2). Between the flask and condenser is then placed a small Soxhlet extractor containing 3.0 g. of dry benzamide-C^{14}. The stirred ether mixture is refluxed for 3 days under nitrogen, slowly extracting the amide into the reaction mixture which is then hydrolyzed with dilute hydrochloric acid. The product is recovered from the ether extract and recrystallized from ethanol-water; yield 4.5 g. (78%), m.p. 132°.

(b) *1-(p-Chlorophenyl)-2-phenylglyoxal-2-C*^{14}, *(C*$_1^{14}$*-4-Chlorobenzil)* (Note 3). A mixture of 1.0 g. of 2-(*p*-chlorophenyl)acetophenone-1-C^{14}, 4 ml. of acetic anhydride and 0.86 g. of selenium dioxide is heated at 140° for 3 hours. The solution is separated with a micropipette, then heated with 5 ml. of water and 0.1 g. of charcoal, filtered and cooled. The yellow product is collected, recrystallized from aqueous alcohol and dried under vacuum; yield 1.2 g. (96%), m.p. 73°.

B. Notes

1. The procedure is a modification of the method of Jenkins[1] for preparing deoxybenzoins. The method fails with the 4-methoxyphenyl Grignard because of preferential coupling of the reagent.

2. The bright green reagent forms in about 1 hour. The substituted benzyl chlorides are prepared by lithium aluminum hydride reduction of the corresponding benzoic acids and treatment of the benzyl alcohols with phosphorus trichloride.

3. See 2-phenylglyoxal-2-C^{14}.

C. Other Preparations

In a similar manner from benzamide-C^{14} and the appropriate Grignard reagent is prepared the series: 2-(*m*-chlorophenyl)acetophenone-1-C^{14} (58.5%, m.p. 44°), 1-(*m*-chlorophenyl)-2-phenylglyoxal-2-C^{14} (95%, m.p. 86°); and 2-(*p*-tolyl)acetophenone-1-C^{14} (38.9%, m.p. 96°), 1-(*p*-tolyl)-2-phenylglyoxal-2-C^{14} (70.2%, m.p. 31°).

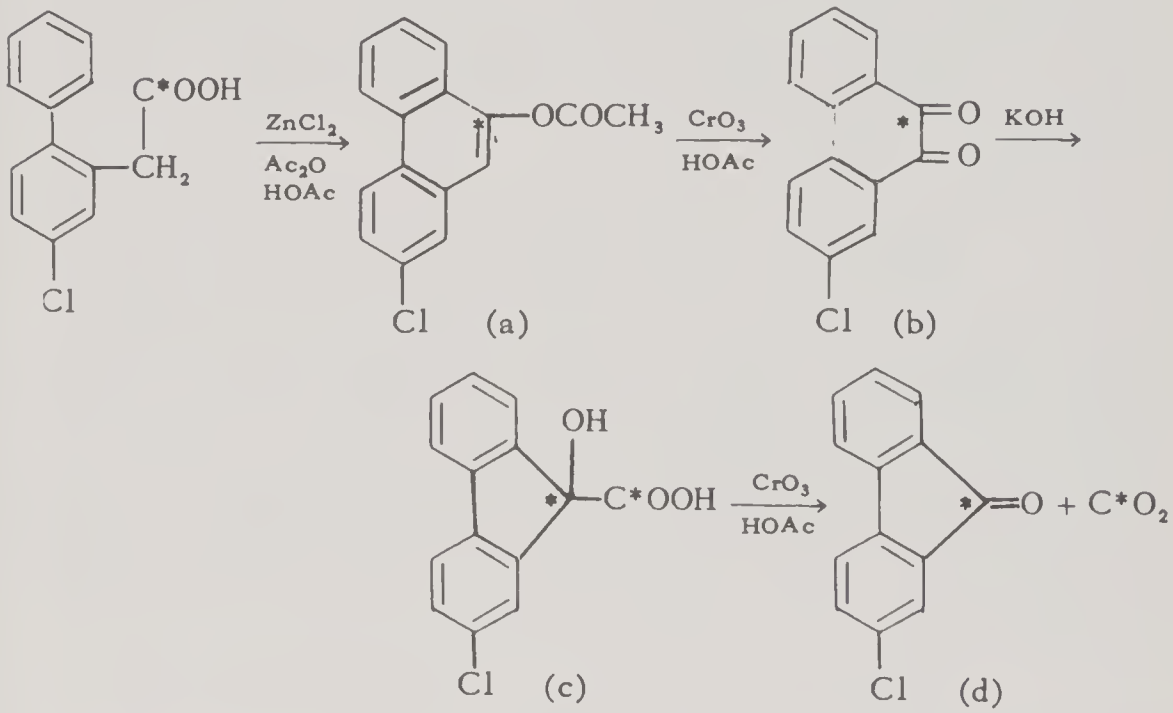
1-(*p*-Methoxyphenyl)-2-phenylglyoxal-C^{14} is prepared by oxidation of 2-(*p*-methoxyphenyl)acetophenone-1-C^{14} with selenium dioxide in dioxane (acetic anhydride gives oils); yield 87%, m.p. 62°.

From *o*-chlorophenylacetic-1-C^{14} acid is prepared[2] 2-(*o*-chlorophenyl)-acetophenone-1-C^{14} (80.5% yield, m.p. 68.5–69.2°), and 1-(*o*-chloro-

phenyl)-2-phenylglyoxal-2-C^{14} (crude yield quantitative, m.p. 41.5–43° from 90% alcohol). See acetophenone-2-C^{14}, Method VI.

[1]S. S. Jenkins, J. Am. Chem. Soc., *55*, 703 (1933).
[2]G. G. Smith and D. G. Ott, J. Am. Chem. Soc., 77, 2342 (1955); *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 156.

2-CHLORO-9-FLUORENONE-9-C^{14}



D. G. Ott and G. G. Smith, J. Am. Chem. Soc., 77, 2325 (1955).

A. Procedure (Note 1)

(a) *2-Chloro-9-phenanthryl-9-C^{14} Acetate* (Note 2). To a hot solution of 3.0 g. (12.1 mmoles) of (4-chloro-2-biphenylyl)acetic-1-C^{14} acid and 11 ml. of acetic anhydride is added a hot solution of 0.94 g. of freshly fused zinc chloride in 11 ml. of acetic acid. The mixture is refluxed for 7 hours; then the excess anhydride is hydrolyzed by the slow addition of a few ml. of water, the hot solution is poured into 75 ml. of water, and the precipitate is extracted with three 15-ml. portions of ether. The combined ether solution is washed with water, cold 8.5% potassium hydroxide solution and water, then dried over magnesium sulfate, decolorized, and evaporated to dryness under reduced pressure. The crude product is recrystallized from methanol; yield 2.75 g. (83%), m.p. 110–111.5°.

(b) *2-Chlorophenanthrenequinone-9-C*^{14}. To a stirred solution of 1.50 g. (5.5 mmoles) of 2-chloro-9-phenanthryl-9-C^{14} acetate and 6 ml. of acetic acid, maintained at 85°, is added 1.75 g. of chromium trioxide in small portions over a period of 1 hour. The mixture is stored overnight at 7° and the product then is collected and washed with acetic acid; yield 1.08 g., 80% (Note 3).

(c) *2-Chloro-9-hydroxy-9-fluorenecarboxylic-*$C^{14}_{1/1}$*-9-*$C^{14}_{1/1}$ *Acid* (Note 4). A solution of 0.20 g. (0.8 mmole) of 2-chlorophenanthrenequinone-9-C^{14} and 20 ml. of 8.5% aqueous potassium hydroxide solution is stirred for 2 hours at 85°. The cooled, filtered solution is acidified with 6*N* hydrochloric acid and extracted with four 10-ml. portions of ether, evaporation of solvent yields the crude product. Degradation studies (see below) indicate that 83% of the radioactivity is located at C-9 due to migration of the substituted ring.

(d) *2-Chloro-9-fluorenone-9-C*^{14} (Note 5). The crude hydroxy acid is heated on a steam-bath for 1 hour with a solution of 0.40 g. of chromium trioxide, 3 ml. of water and 6 ml. of acetic acid, the evolved carbon-C^{14} dioxide being swept into an alkali trap with a carbon dioxide-free air stream. The mixture is diluted with 50 ml. of water, and the precipitate is extracted into three 20-ml. portions of ether. The extracts are washed with water, 3 *N* sodium hydroxide and water, then dried over magnesium sulfate, decolorized with charcoal and evaporated to dryness. The yield of crude product is 0.16 g., 93% (Note 6). The molar specific activity is about 83% of that of the 2-chloro-9-hydroxy-9-fluorenecarboxylic-$C^{14}_{1/1}$-9-$C^{14}_{1/1}$ acid.

B. Notes

1. These preparations are incidental to a study of the alkaline rearrangement of chlorophenanthrenequinones.

2. The method of ring closure is adapted from that of Fieser and Hershberg.[1]

3. Two recrystallizations from acetic acid give orange-red needles melting at 240–241°.

4. The alkaline rearrangement of phenanthrenequinone was first reported by Baeyer.[2] Since the alcohols used as solvents in the rearrangement of benzils reduce the phenanthrenequinones to the quinhydrones, the rearrangements are carried out in aqueous media. The additional bond present in the phenanthrenequinones has little effect in determining the course of the rearrangement; see 1-(*p*-chlorophenyl)glyoxal-2-C^{14}.

5. This compound is the same as that obtained from 7-chlorophenanthrenequinone-9-C^{14}.

6. The bright yellow needles, obtained by two recrystallizations from methanol, melt at 124–124.5°.

C. Other Preparations

The following isomeric series of compounds is prepared by the same procedure, starting from the appropriate acid:

(3-Chloro-2-biphenylyl)acetic-1-C^{14} acid:[3]

1-Chloro-9-phenanthryl-9-C^{14} acetate,[3] 86%, m.p. 119–120°. Hydrolysis with ethanolic potassium hydroxide forms 1-chloro-9-phenanthrol-9-C^{14}, 99.5%, m.p. 211–212°. The phenanthrols are subject to air oxidation.

1-Chlorophenanthrenequinone-9-C^{14},[3] 27%, m.p. 228–230°.

1-Chloro-9-fluorenone-9-C^{14},[3] 92%, m.p. 139.5–140°; the molar specific activity is 1.6% of that of the starting material.

(5-Chloro-2-biphenylyl)acetic-1-C^{14} acid:

3-Chloro-9-phenanthryl-9-C^{14} acetate, 46%, m.p. 114–116°.

3-Chlorophenanthrenequinone-9-C^{14}, 65%, m.p. 259–260°.

3-Chloro-9-fluorenone-9-C^{14}, 56%, m.p. 157–158°. It is necessary to add dioxane to the rearrangement mixture to increase the reaction velocity. The 3-chloro-9-hydroxy-9-fluorenecarboxylic-$C^{14}_{1/1}$-9-$C^{14}_{1/1}$ acid partially undergoes spontaneous decarboxylation and oxidation to the fluorenone during the rearrangement step. The molar specific activity is 67% of that of the starting material.

(4′-Chloro-2-biphenylyl)acetic-1-C^{14} acid:

7-Chloro-9-phenanthryl-9-C^{14} acetate, 30%, m.p. 132–132.5°.

7-Chlorophenanthrenequinone-9-C^{14}, 67%, m.p. 240–241°.

2-Chloro-9-fluorenone-9-C^{14}, 94%, m.p. 124–124.5°; the molar specific activity is about 20% of that of the starting material, whereas the value is 83% when the compound is prepared from (a).

2-Biphenylylacetic-1-C^{14} acid:

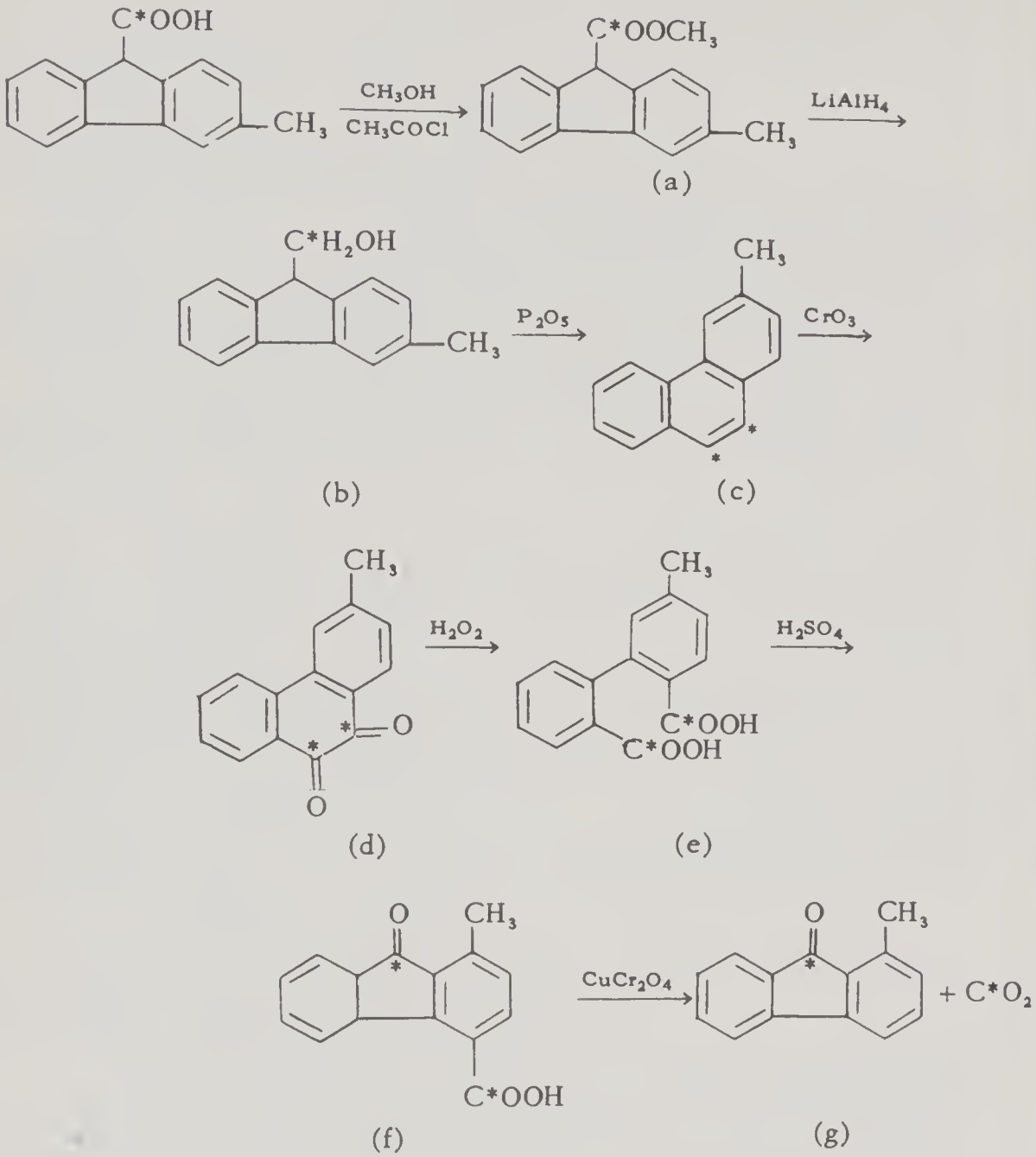
9-Phenanthryl-9-C^{14} acetate, 71%, m.p. 74–75°.

Phenanthrenequinone-9-C^{14}, 50%, m.p. 202–204°.

9-Fluorenone-9-C^{14}, 90%, m.p. 83.5–84°; the molar specific activity is about 52% of that of the starting material. The preferential migration of the substituted ring is attributed to isotope effect only.

[1]L. F. Fieser and E. B. Hershberg, J. Am. Chem. Soc., 59, 1028 (1937).
[2]A. Baeyer, Ber., *10*, 123 (1877).
[3]G. G. Smith and D. G. Ott, J. Am. Chem. Soc., 77, 2342 (1955).

1-METHYL-9-FLUORENONE-9-C^{14}



C. J. Collins and B. M. Benjamin, J. Am. Chem. Soc., 75, 1644 (1953).

A. Procedure (Note 1)

(a) *Methyl 3-Methyl-9-fluorenecarboxylate-C^{14}.* A cooled solution of 1 ml. of acetyl chloride in 30 ml. of methanol is added to a flask containing 0.288 g. of 3-methyl-9-fluorenecarboxylic-C^{14} acid. After 20 minutes the mixture is distilled under vacuum; yield quantitative, 0.305 g. (Note 2).

(b) *3-Methyl-9-fluorenemethanol-α-C^{14}.* A clear saturated solution of lithium aluminum hydride in ether is added to 0.305 g. of methyl 3-methylfluorene-9-carboxylate-C^{14}. A violent evolution of gas takes place,

and the excess reagent is then decomposed with ethyl acetate, followed by dilute hydrochloric acid. Extraction with ether and evaporation of solvent yield 0.311 g. (theory, 0.270 g.) of crude product (Note 3).

(c) *3-Methylphenanthrene-9,10-*$C^{14}_{1/2}$. A mixture of 0.508 g. of crude 3-methyl-9-fluorenemethanol-α-C^{14}, 20 ml. of dried xylene and 0.20 g. of phosphorus pentoxide is refluxed for 30 minutes. The mixture is treated with water, and the organic layer is separated and evaporated under vacuum, yielding 0.560 g. of oil (Note 4). Degradation studies (see below) indicate that 73% of the activity is at C-10.

(d) *3-Methylphenanthrenequinone-9,10-*$C^{14}_{1/2}$. 3-Methylphenanthrene-9,10-$C^{14}_{1/2}$ (1.5 g.) is oxidized in acetic acid solution (75 ml.) with 6.9 g. of potassium dichromate according to the procedure of Haworth,[1] which follows. The yield of crude product is 1.15 g., 66% (Note 5).

A solution of 2.5 parts of chromic anhydride in 4 volumes of acetic acid and 2 volumes of water is added gradually to a solution of 1 part of the hydrocarbon in 5 volumes of acetic acid at 60–70°. The mixture is heated for 1 hour on a water-bath, then diluted with water, and the product is collected, washed with ether and recrystallized from alcohol.

(e) *5-Methyldiphenic-*$C^{14}_{1/2}$ *Acid.* The crude 3-methylphenanthrenequinone-9,10-$C^{14}_{1/2}$ (1.06 g.) is oxidized with hydrogen peroxide according to the procedure of Kruber and Marx,[2] which follows; yield 0.956 g., (78%), m.p. 201–202°.

A mixture of 2 g. of 3-methyl-9,10-phenanthrenequinone, 10 ml. of 30% hydrogen peroxide and 18 ml. of 2 *N* sodium hydroxide is stirred well for 1.5 hours until the vigorous frothing subsides. The mixture is warmed for 1 hour on a water-bath, diluted with water, stored overnight, filtered and acidified. The product settles out in colorless flocks; yield 1.7 g., m.p. 208° after recrystallization from hot water.

(f) *1-Methyl-9-oxo-4-fluorenecarboxylic-*$C^{14}_{1/1}$*-9-*$C^{14}_{1/1}$ *Acid.* The unpurified 5-methyldiphenic-$C^{14}_{1/2}$ acid is dissolved in 100 ml. of cold sulfuric acid and set aside for 18 hours. The reaction mixture is poured onto ice and subjected to continuous ether extraction; crude yield 1.12 g. (Note 6). Two recrystallizations from acetic acid give 0.460 g. (53%) of pure product, m.p. 210–212°. The molar specific activity is about the same as that of the starting material.

(g) *1-Methyl-9-fluorenone-9-*C^{14}. A mixture of 0.434 g. of 1-methyl-9-oxo-4-fluorenecarboxylic-$C^{14}_{1/1}$-9-$C^{14}_{1/1}$ acid, 20 ml. of quinoline and 0.420 g. of copper chromite catalyst is refluxed for 1 hour (Note 7). The solution is filtered, acidified with hydrochloric acid and subjected to continuous ether extraction; yield 0.302 g. (85%). The melting point is 98–99° after two recrystallizations from dilute alcohol. The molar specific activity is about 27% of that of the starting material, thus showing the contribution of C-9 of the hydrocarbon (c).

B. Notes

1. These preparations are incidental to a tracer study of steric effects in the Wagner rearrangement.

2. Recrystallization from hexane gives material melting at 81–81.5°.

3. Recrystallization from hexane and from dilute methanol gives material melting at 96–98°.

4. In a trial run, the oil gave crystalline product (88% yield), m.p. 58–59.5°.

5. Recrystallization from acetic acid gives material melting at 208–210°.

6. Since the theoretical yield is only 0.877 g., it is presumed that some sulfonation takes place during cyclization.

7. The evolved carbon-C^{14} dioxide is swept into a barium hydroxide trap.

8. The xylene solution is washed with alkali and evaporated, and the crude yellow product is purified by passage in benzene solution through a column of alumina.

C. Other Preparations

In a similar manner from 1-methyl-9-fluorenecarboxylic-C^{14} acid is prepared[3] the series: methyl 1-methyl-9-fluorenecarboxylate-C^{14} (*via* diazomethane, yield 100%, m.p. 95–96° from cyclohexane), 1-methyl-9-fluorenemethanol-α-C^{14} (yield 100%, Note 8, m.p. 85.5–86.4° from cyclohexane), 1-methylphenanthrene-9,10-$C^{14}_{1/2}$ (yield 100%, m.p. 119.5–120.5° from alcohol; 50% activity at C-9), 1-methylphenanthrenequinone-9,10-$C^{14}_{1/2}$, 3-methyldiphenic-$C^{14}_{1/2}$ acid (yield 85%, m.p. 190–191° from chloroform), 3-methyl-9-oxofluorene-4-carboxylic-$C^{14}_{1/1}$-9-$C^{14}_{1/1}$ acid (yield 77%, m.p. 206° from benzene; molar specific activity same as that of the starting materials), and 3-methyl-9-fluorenone-9-C^{14} (crude yield 0.069 g. based on 0.063 g. of the keto acid). As the product cannot be crystallized, it is converted to the oxime, m.p. 185° from cyclohexane. Directions are given by Benjamin[3] for degrading the product with permanganate to 9-oxofluorene-3-carboxylic-9-C^{14} acid and 4-carboxy-C^{14}-isophthalic acid; yields are small.

3-Methyl-9-fluorenone-9-C^{14} may be formed, presumably, by distillation of 5-methyldiphenic-$C^{14}_{1/2}$ acid with soda lime.[4]

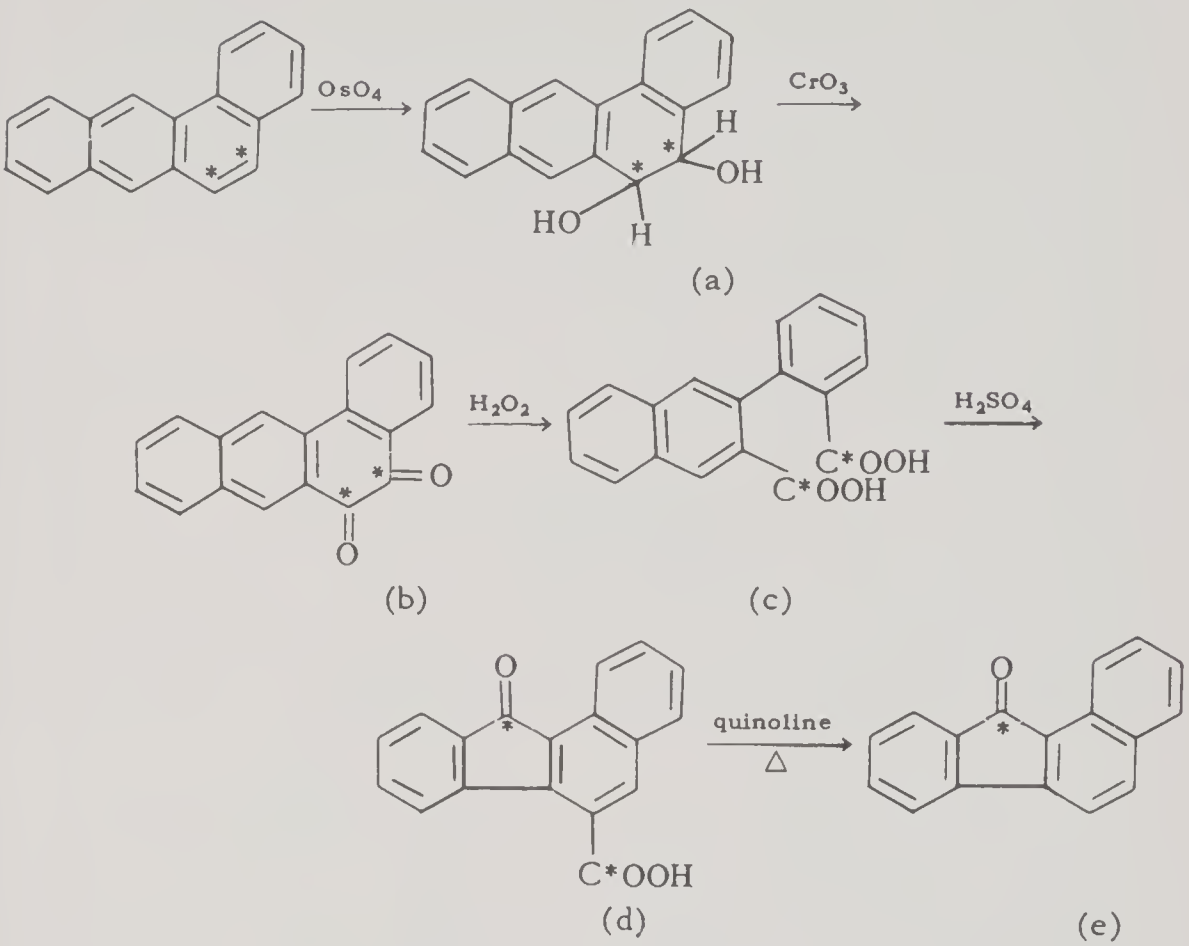
[1] R. D. Haworth, J. Chem. Soc., *1932*, 1125.

[2] O. Kruber and A. Marx, Ber., *71*, 2478 (1938).

[3] B. M. Benjamin and C. J. Collins, J. Am. Chem. Soc., *75*, 402 (1953).

[4] C. J. Collins and B. M. Benjamin, J. Am. Chem. Soc., *75*, 1644 (1953); O. Kruber, A. Marx and W. Schade, Ber., *71B*, 2478 (1938).

11-CHRYSOFLUORENONE-11-C^{14}



C. J. Collins, J. G. Burr, Jr. and D. N. Hess, J. Am. Chem. Soc., 73, 5176 (1951).

A. Procedure

(a) *5,6-Dihydrobenz*[a]*anthracene-5,6-diol-5,6-*$C^{14}_{1/2}$. Benz[*a*]anthracene-5,6-$C^{14}_{1/2}$ (0.930 g.) is dissolved in 40 ml. of benzene in a 250-ml., glass-stoppered bottle. A 1-g. vial of osmium tetroxide is broken in the bottle, 1 ml. of pyridine is added, the bottle is stoppered, and the mixture is stirred (magnetically)for 1 hour and set aside for 2 days. Methylene chloride (50 ml.) and 100 ml. of 3% potassium hydroxide, containing 10 g. ot mannitol, are added, and the bottle is shaken for 5 hours. The organic layer is separated,and evaporated at room temperature in an air stream, and the residue is taken up in a benzene-chloroform solution and filtered. The filtrate is evaporated, and the resulting residue is crystallized from chloroform; yield 0.773 g. (74%), m.p. 205–206°.

(b) *Benz*[a]*anthracene-5,6-dione-5,6-*$C^{14}_{1/2}$. A solution of 0.733 g. of 5,6-dihydrobenz[*a*]anthracene-5,6-diol-5,6-$C^{14}_{1/2}$ is mixed with a solution of 0.78 g. of chromium trioxide in 1.5 ml. of water and 13.5 ml. of acetic acid. After standing for 15 minutes, the mixture is chilled and filtered; yield 0.432 g. (66%), m.p. 263–265° (Note 1).

(c) *3-(o-Carboxy-$C^{14}_{1/1}$-phenyl)-2-naphthoic-$C^{14}_{1/1}$ Acid*, (*2-Phenylnaphthalene-2′,3-dicarboxylic-$C^{14}_{1/2}$ Acid*). To a solution of 0.688 g. of benz[*a*]anthracene-5,6-dione-$C^{14}_{1/2}$ in 70 ml. of acetic acid, heated on a steam-bath, is added 0.45 ml. of 30% hydrogen peroxide. A similar addition of peroxide is made at 1 hour, and heating is continued for another hour. After standing overnight, the mixture is treated with a third addition of peroxide, and evaporated on the steam-bath in an air stream. The rexidue is washed with water and dissolved in warm sodium bicarbonate solution; filtration removes 0.221 g. of an orange powder. The cold filtrate is acidified with concentrated hydrochloric acid, and the precipitated product is collected and air-dried; yield 0.445 g. (61%), m.p. about 130° (Note 2).

(d) *11-Oxo-6-chrysofluorenecarboxylic-$C^{14}_{1/1}$-11-$C^{14}_{1/1}$ Acid.* A solution of 0.430 g. of 3-(*o*-carboxy-$C^{14}_{1/1}$-phenyl)-2-naphthoic-$C^{14}_{1/1}$ acid in 40 ml. of 98% sulfuric acid is allowed to stand for 2 hours and then poured onto ice. The brown precipitate is dissolved in boiling toluene and filtered from a residue of about 0.1 g. Concentration of the solution to 20 ml. produces a 39% yield of amorphous orange powder, m.p. 250–255° (Note 3).

(e) *11-Chrysofluorenone-11-C^{14}*, (*11H-Benzo*[a]*fluoren-11-one-11-C^{14}*). The keto acid (0.160 g.) is decarboxylated by heating under reflux for 15 minutes with 15 ml. of quinoline and 0.200 g. of basic copper carbonate (Note 4). The reaction mixture is acidified with dilute hydrochloric acid, and the orange precipitate is extracted into ether and benzene. The dried extract is evaporated, and a benzene solution of the residue is passed through an alumina column. Evaporation of the eluate yields 0.112 g. (90%) of product. Recrystallation from pentane gives 0.076 g. of orange needles melting at 132–133° (Note 5).

B. Notes

1. By treating the mother liquor and residual material from the diol preparation with 0.5 g. of chromium trioxide an additional 0.255 g. of product is obtained.

2. A sample is dissolved in hot dilute methanol and decanted from a residual tar, then chilled and set aside to produce needles melting at 243–246°.[1]

3. Recrystallization of 0.240 g. of the acid from toluene gives 0.168 g. of yellow needles, m.p. 267–268°. The molar specific activity is 98.5% of that of the hydrocarbon starting material, and degradation establishes the activity of the carbonyl and carboxyl groups to be nearly equal.

4. The evolved radioactive gases are collected in alkali.

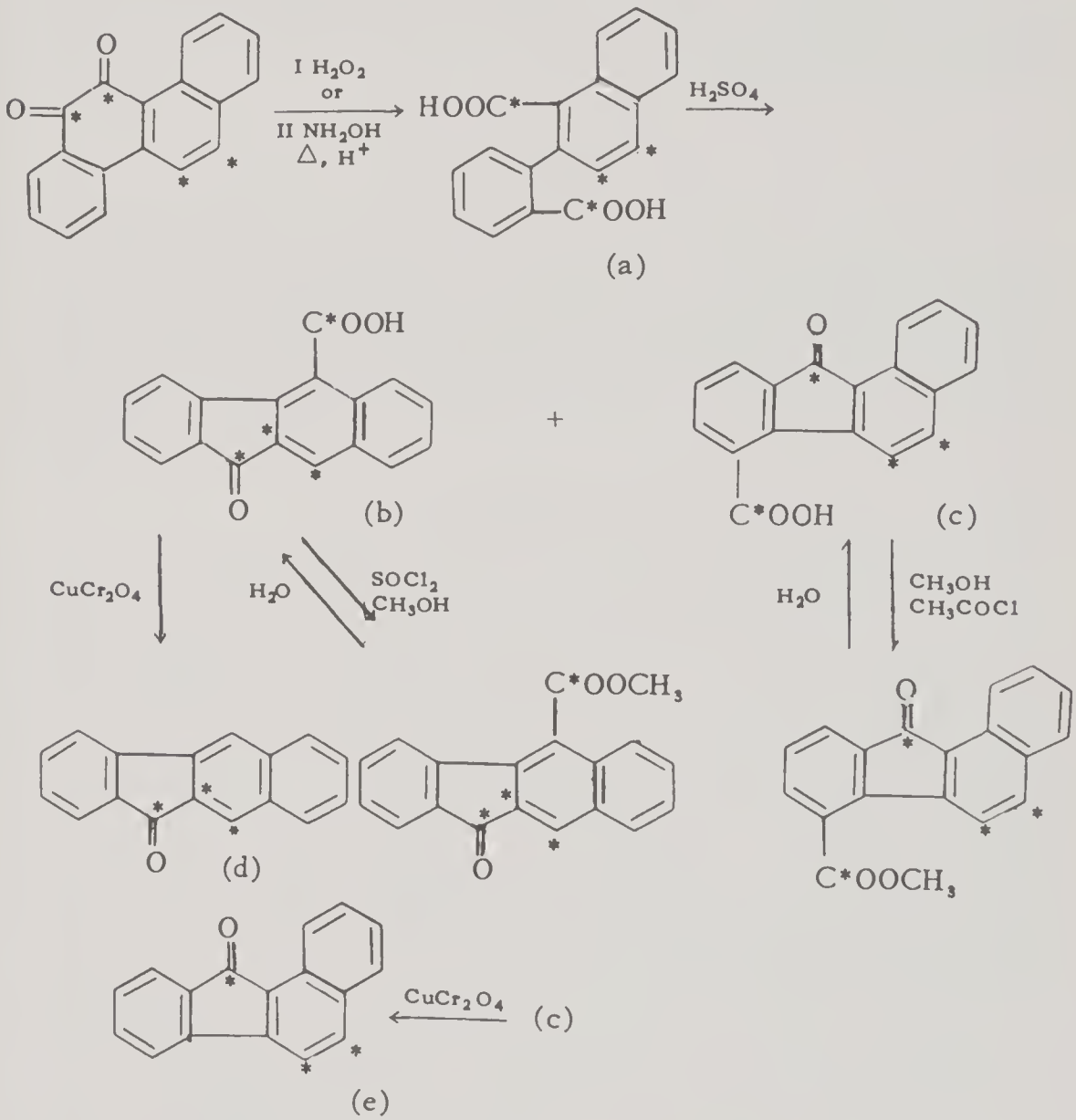
5. There is no melting point depression upon mixed melting with an authentic sample.[2] The specific activity of the ketone indicates that 48% of the activity of the starting hydrocarbon is found at C-5.

C. Other Preparations

11-Chrysofluorenone-11-C^{14} and 11-oxo-6-chrysofluorenecarboxylic-$C^{14}_{1/1}$-11-$C^{14}_{1/1}$ acid have been formed in small yield, by heating 3-(*o*-carboxy-$C^{14}_{1/1}$-phenyl)-2-naphthoic-$C^{14}_{1/1}$ acid.

[1]L. F. Fieser and E. M. Dietz, J. Am. Chem. Soc., *51*, 3141 (1929).
[2]M. Orchin and L. Reggel, *ibid*, 73, 439 (1951).

11-CHRYSOFLUORENONE-5,6,11-$C^{14}_{1/3}$



C. J. Collins, D. N. Hess, R. H. Mayor, G. M. Toffel and A. R. Jones, J. Am. Chem. Soc., 75, 397 (1953).

A. Procedure

(a) 2-(o-*Carboxy*-$C^{14}_{1/1}$-*phenyl*)-1-*naphthoic*-$C^{14}_{1/1}$-3,4-$C^{14}_{1/2}$ *Acid*, (*2-Phenylnaphthalene*-3,4-$C^{14}_{1/2}$-1,2′-*dicarboxylic*-$C^{14}_{1/2}$ *Acid*).

METHOD I

The mono-oxime is prepared from 3.0 g. of 5,6-chrysenedione-5,6,11,12-$C^{14}_{1/4}$, 1 g. of hydroxylamine hydrochloride, 15 ml. of ethanol and 2 ml. of pyridine (Note 1); yield 3.09 g. (96%), m.p. 165–166°. The Beckmann rearrangement of 4.50 g. of oxime at 130–140° yields 2.81 g. (55.8%) of mixed amido acids.[1] This mixture (2.06 g.) is hydrolyzed by heating for 6 hours at 150° in a sealed tube with 60 ml. of acetic acid and 15 ml. of hydrochloric acid (Note 2). The reaction mixture is evaporated to dryness in an air stream. From the acidified bicarbonate-soluble fraction of this residue (Note 3) is isolated, by continuous ether extraction, 1.341 g. (70%) of product (Note 4).

METHOD II

To a refluxing mixture of 9.61 g. of 5,6-chrysenedione-5,6,11,12-$C^{14}_{1/4}$ and 700 ml. of acetic acid is added 10 ml. of 30% hydrogen peroxide. At 1 hour, a similar amount of peroxide is added and heating is continued for another hour. The mixture is cooled and set aside overnight, then a last portion of peroxide is added and heating is resumed for 1 hour. The cooled mixture is poured into water, and the precipitate is collected, digested in 5% sodium carbonate solution and filtered. From the filtrate is obtained 5.3 g. (49%) of crude product (Note 5).

(b) *11-Oxo-11*H*-benzo*[b]*fluorene-5-carboxylic-*$C^{14}_{1/1}$*-10,10a,11-*$C^{14}_{1/3}$ *Acid.* Crude 2-(*o*-carboxy-$C^{14}_{1/1}$-phenyl)-1-naphthoic-$C^{14}_{1/1}$-3,4-$C^{14}_{1/2}$ acid (4.19 g.) is cyclized, by the procedure of Graebe and Gnehm,[2] by standing at room temperature for 12–24 hours mixed with 33 ml. of concentrated sulfuric acid. The yield of mixed keto acids (b) and (c) is 1.93 g., 49% (Note 6). Purification is effected through esterfication in the following manner. To the mixed acids is added a cold solution of 1 ml. of acetyl chloride in 175 ml. of methanol, and the mixture is refluxed for 24 hours. The solvent is removed in an air stream, and an ether solution of the residue is extracted several times with sodium bicarbonate solution (Note 7). Acidification of the alkaline extracts yields 1.06 g. of crude product (Note 8). The acid is refluxed for 2 hours with 2 ml. of thionyl chloride in 20 ml. of benzene; then the mixture is evaporated to dryness. The residue is refluxed for 1.5 hours with 0.5 ml. of pyridine in 20 ml. of methanol; then the solution is evaporated to dryness. An ether solution of the residue is washed with sodium carbonate solution, then evaporated, leaving 1.01 g. (3.51 mmoles, 50%) of methyl 11-oxo-11*H*-benzo[b]fluorene-5-carboxylate-$C^{14}_{1/1}$-10,10a, 11-$C^{14}_{1/3}$ (Note 9). The ester is hydrolyzed by refluxing for 24 hours with 3 g. of sodium hydroxide in 15 ml. of water and 60 ml. of methanol. The solution is concentrated almost to dryness and acidified with dilute hydrochloric acid; yield 0.54 g. (Note 10). The molar specific activity is unchanged from that of the chrysenequinone.

(c) *11-Oxo-7-chrysofluorenecarboxylic-$C^{14}_{1/1}$-5,6,11-$C^{14}_{1/3}$ Acid*), (*11-Oxo-11H-benzo*[a]*fluorene-7-carboxylic-$C^{14}_{1/1}$-5,6,11-$C^{14}_{1/3}$* Acid). The ether solution from the methanol-acetyl chloride esterification (Note 7) is evaporated, giving 0.910 g. of methyl 11-oxo-7-chrysofluorenecarboxylate-$C^{14}_{1/1}$-5,6,11-$C^{14}_{1/3}$ (3.16 mmoles, 45%), m.p. 169–173° (Note 11). The ester (0.660 g.) is hydrolyzed as above, yielding 0.473 g. of the acid (Note 12). The molar specific activity is about the same as that of the chrysenequinone.

(d) *11H-Benzo*[b]*fluoren-11-one-10,10a,11-$C^{14}_{1/3}$*. A mixture of 0.326 g. of 11-oxo-11*H*-benzo[b]fluorene-5-carboxylic-$C^{14}_{1/1}$-10,10a,11-$C^{14}_{1/3}$ acid, 10 ml. of quinoline and 0.020 g. of copper chromite catalyst is heated at 250° (Note 13). The filtered reaction mixture is taken to dryness, 0.232 g. (84%). The yield of recrystallized product is 0.188 g. (73%), m.p. 156–157°, and the molar specific activity is 62% of that of the starting material (Note 14).

(e) *11-Chrysofluorenone-5,6,11-$C^{14}_{1/3}$*, (*11H-Benzo*[a]*fluoren-11-one-5,6,11-$C^{14}_{1/3}$*). 11-Oxo-7-chrysofluorenecarboxylic-$C^{14}_{1/1}$-5,6,11-$C^{14}_{1/3}$ acid (0.225 g.) is decarboxylated in the manner described above. The yield is 0.172 g. (Note 15), and the molar specific activity is 88% of that of the starting material (Note 14).

B. Notes

1. The use of pyridine is an improvement over the original method of Graebe and Gnehm.[2]

2. The tube is sealed under vacuum, while the mixture is frozen. Alkaline hydrolysis gives incomplete deamination.

3. From the bicarbonate-insoluble fraction is obtained 0.436 g. (26.8%) of 11-chrysofluorenone-5,6,11-$C^{14}_{1/3}$, m.p. 131–133°. When the hydrolysis is effected at 200°, the yield is quantitative. This ketone contains a different isotopic distribution in the three positions than does the ketone prepared by decarboxylation of 11-oxo-7-chrysofluorenecarboxylic-$C^{14}_{1/1}$-5,6,11-$C^{14}_{1/3}$ acid. The molar specific activity is 64.5%, instead of 88.3%, of that of the intermediate 2-(*o*-carboxy-$C^{14}_{1/1}$-phenyl)-1-naphthoic-$C^{14}_{1/1}$-3,4-$C^{14}_{1/2}$ acid. A different mechanism is postulated for its formation; i.e., decarboxylation of the naphthoic acid group.

4. Two recrystallizations from dilute alcohol give material melting at 209.4–210.4° (cor.).

5. The crude product is used directly for cyclization, since purification of the resulting keto acids is much simpler; repeated reprecipitation from alkaline solution and recrystallization from chloroform and methanol give a pure sample of (a).

6. When purified samples of dicarboxylic acid are cyclyzed, the yield runs as high as 75%. As much as an additional 15% of 11-chrysofluorenone-5,6,11-$C^{14}_{1/3}$ occurs as a bicarbonate-insoluble fraction.

7. The ether solution contains the methyl ester of (c) only.

8. The keto acid (b) contains impurities which are best removed by esterification, followed by hydrolysis back to the acid, as described.

9. The melting point is 155–156° after recrystallization from dilute methanol.

10. The melting point is 255–256° after crystallization from acetone-water, or acetic acid.

11. Recrystallization from dilute methanol produces orange needles, m.p. 175–176°.

12. Recrystallization several times from acetic acid gives material melting at 283–284°.

13. The evolution of carbon-C^{14} dioxide takes place between 15 and 35 minutes.

14. The calculation of the contribution of C-5 and C-6 to the specific activity must allow for their identity with C-11 and C-12, respectively, in the chrysene structure.

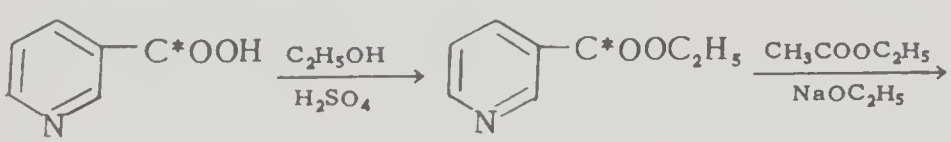
$$C_5 + C_6 = \text{relative sp. act. of starting material} = 100\%$$
$$\tfrac{1}{2}C_5 + C_6 = \text{relative sp. act. of (d)} = 62\%$$
$$C_5 + \tfrac{1}{2}C_6 = \text{relative sp. act. of (e)} = 88.2\%$$

Since $C_5 = 76\%$, there is greater 1-naphthyl migration than there is phenyl migration, during the Wagner rearrangement that results in the formation of the chrysene-5,6-$C^{14}_{1/2}$ from which the quinone is made; see 5,6-chrysenedione-5,6,11,12-$C^{14}_{1/4}$.

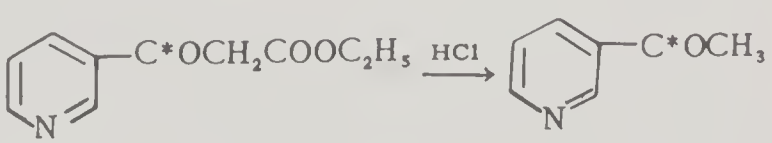
15. A purified sample melts at 135–136°.

[1]C. Graebe and F. Honigsberger, Ann., *311*, 257 (1900).
[2]C. Graebe and R. Gnehm, Jr., Ann., *335*, 113 (1904).

METHYL 3-PYRIDYL KETONE-C^{13}
(3-Acetyl-1-C^{13}-pyridine)



(a)



(b) (c)

W. T. Beher, W. M. Holliday and O. H. Gaebler, J. Biol. Chem., *198*, 573 (1952).

A. Procedure

(a) *Ethyl Nicotinate-C^{13}*. The esterification of 5.88 g. of nicotinic-C^{13} acid is effected by an adaptation of the methods of Gilman[1] and Burrus,[2] which follow. The yield is 89%, based on the nicotinic-C^{13} acid not recovered by exhaustive ether extraction at pH 3; the recovery of starting material is 13%.

Nicotinic acid (100 g.) is dissolved in a mixture of 250 ml. of ethanol and 125 ml. of concentrated sulfuric acid by warming on a steam-bath. The solution is cooled in an ice-bath, poured into a mixture of 2 kg. of cracked ice and 350 g. of potassium carbonate, and filtered. The filtered. The filtrate is saturated with sodium carbonate and extracted with ether. The extract is dried and concentrated, and the product is distilled; yield 88 g. (72%), b.p. 107–108° (16 mm.).

(b) *Ethyl β-Oxo-3-pyridinepropionate-β-C^{13}*. The Claisen condensation of the ester with ethyl acetate is effected by the procedure of Gilman,[1] which follows. The product is not isolated, but hydrolyzed directly to the ketone.

To a suspension of 0.75 mole of sodium ethoxide in 690 ml. of benzene (Note 1) is added slowly, under reflux, a mixture of 88 g. (1 mole) of anhydrous ethyl acetate and 75.5 g. (0.5 mole) of ethyl nicotinate. The mixture is refluxed for twelve hours, by which time it becomes clear (Note 2).

(c) *Methyl 3-Pyridyl Ketone-C^{13}*. The benzene solution of the condensation product is treated with an excess of dilute hydrochloric acid, the benzene is distilled off on a steam-bath, and the β-keto ester is subjected to hydrolytic cleavage by refluxing the aqueous acidic solution for 2.5 hours. The solution is made basic and exhaustively extracted with ether in a continuous extractor. The solvent is evaporated and the residue is distilled. The yield of product is 95%, if allowance is made for recovery of nicotinic acid (17%) from the alkaline solution (Note 3); b.p. 93° (7 mm.).

Methyl 3-pyridyl ketone-C^{13} hydrochloride is prepared by treating a benzene solution of the free base with benzene saturated with hydrogen chloride. The compound melts at 180–181°, after two recrystallizations from methanol-ether.

B. Notes

1. The stoichiometric amount of absolute ethanol is added dropwise to a mixture of sodium-sand and dry benzene, at such a rate as to promote gentle reflux until reaction is complete.

2. The isolation of ethyl β-oxo-3-pyridinepropionate is described by Gilman;[1] yield 67%, b.p. 121–123° (0.4 mm.).

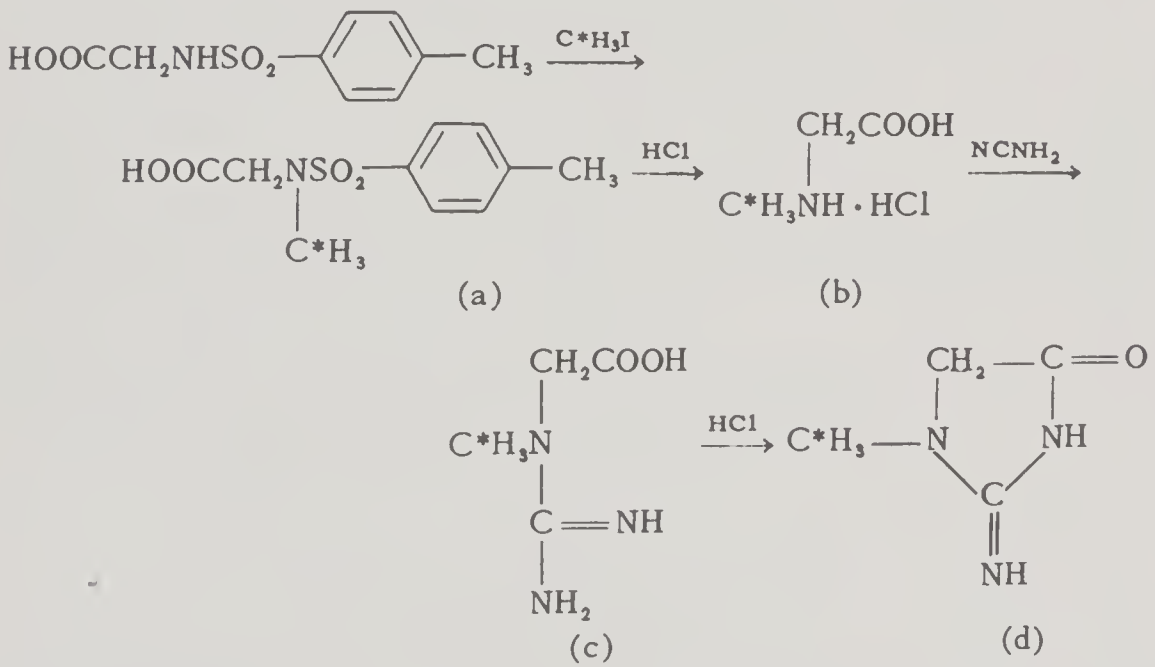
3. Burrus[2] has reported a yield of 85% by this procedure, b.p. 80° (5 mm.).

C. Other Preparations

Ethyl nicotinate-C^{14} has been prepared by Dawson[3] (90% yield) by treating an ethereal suspension of nicotinic-C^{14} acid with an ethereal solution of diazoethane until no further evolution of nitrogen was observed. The solution was evaporated, and the product was distilled at less than 0.1 mm.

[1]H. Gilman and H. S. Broadbent, J. Am. Chem. Soc., *70*, 2755 (1948).
[2]H. O. Burrus and G. Powell, *ibid.*, *67*, 1468 (1945).
[3]R. F. Dawson, D. R. Christman and R. C. Anderson, Atomic Energy Commission Report, BNL-1477.

2-IMINO-1-METHYL-C^{14}-4-IMIDAZOLIDINONE (C_1^{14}-Creatinine)



C. G. Mackenzie and V. du Vigneaud, J. Biol. Chem., *185*, 185 (1950).

A. Procedure

(a) N-*Methyl-*C^{14}-N-p-*toluenesulfonylglycine,* (C_1^{14}-N-p-*Toluenesulfonylsarcosine*). *N-p*-Toluenesulfonylglycine[1] is condensed with methyl-C^{14} iodide by heating in 3 *N* sodium hydroxide at 67° for 1 hour, according to the procedure of Fischer and Bergmann.[1] The product is obtained as platelets from hot water, m.p. 150–152° (cor.).

(b) N-*Methyl-*C^{14}*-glycine Hydrochloride,* (C_1^{14}*-Sarcosine Hydrochloride*). The *N*-methyl-C^{14}-N-*p*-toluenesulfonylglycine is hydrolyzed by heating

with concentrated hydrochloric acid in a sealed tube at 100° for 22 hours, according to the procedure of Fischer and Bergmann;[1] yield 91%.

(c) N-*Amidino*-N-*methyl*-C^{14}-*glycine*, (C_1^{14}-*Creatine*). To a solution of 0.500 g. of *N*-methyl-C^{14}-glycine hydrochloride, 1.6 ml. of water and 0.45 ml. of concentrated ammonium hydroxide is added 1.2 ml. of 30% cyanamide solution, and the mixture is stored for 3 days at room temperature, then 2 days in a refrigerator (Note 1). After cooling in an ice-bath, the product is separated, washed with five small portions each of ice water, ice-cold ethanol and ether, then air-dried. The yield is 65% and the purity is 100.2%, according to the Jaffe reaction (Note 2); anhydrous C_1^{14}-creatine is formed by heating at 105° (Note 3).

(d) *2-Imino-1-methyl*-C^{14}-*4-imidazolidinone*, (C_1^{14}-*Creatinine*). The intramolecular condensation of *N*-amidino-*N*-methyl-C^{14}-glycine, with loss of water, is effected by boiling a hydrochloric acid solution according to the procedure of Edgar and Hinegardner.[2] The yield is about 80%.

B. Notes

1. Crystals of creatine monohydrate appear on the second day.

2. The intensity of the red color produced[3] by the addition of picric acid and sodium hydroxide solution is dependent upon the quantity of creatinine present.

3. The loss in weight corresponds to the theoretical amount. One gram of the monohydrate dissolves in 75 ml. of water, or about 9 l. of alcohol. A mixture of creatine and creatinine is formed in aqueous solution, the equilibrium[3] being determined by the pH.

[1]E. Fischer and M. Bergmann, Ann., *398*, 96 (1913); Chem. Abstracts, 7, 2541 (1913).

[2]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, *1941*, p. 172.

[3]G. Edgar and H. E. Shiver, J. Am. Chem. Soc., 47, *1179 (1925)*.

5,5-DIPHENYLHYDANTOIN-4-C^{14}
(C_1^{14}-Dilantin)

$$(C_6H_5)_2CO + NaC^*N \xrightarrow[CH_3CONH_2]{(NH_4)_2CO_3} (C_6H_5)_2C(NH)C(=O)NHC^*O$$

A. Murray III, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, unpublished work.

A. Procedure (Note 1)

A magnetically stirred mixture of 6.0 mmoles of sodium cyanide-C^{14} (Note 2) and 2.12 g. (11.6 mmoles) of benzophenone is dissolved in 24.0 g. of acetamide by warming under an infrared lamp (Note 3). The mixture is cooled, treated with 1.56 g. (16.1 mmoles) of powdered ammonium carbonate, sealed quickly in a steel bomb (Note 4) and heated at 110° for 7.5 days. The mixture is dissolved in water, acidified with hydrochloric acid, stirred and warmed to evolve any unreacted hydrogen cyanide-C^{14}, then is strongly basified with sodium hydroxide and continuously extracted for a few hours with chloroform to remove the acetamide and excess ketone (Note 5). The solution is acidified to pH 2 with hydrochloric acid, and the precipitate is collected by chloroform extraction. The extract is evaporated, the residue is dissolved in 10 ml. of water with a few drops of saturated sodium hydroxide, and the solution is filtered through Norit, strongly acidified and cooled. The product is collected, washed with water and dried at 100°; yield 1.1112 g. (73.5%), m.p. 286–288° (Note 6).

B. Notes

1. The procedure is an adaptation of Henze's[1] modification of the Bucherer process; also see valine-3-C^{13}.

2. Sodium cyanide-C^{14} solution, transferred with water and 3 drops of saturated sodium hydroxide solution to a Pyrex liner for a steel bomb, is evaporated under reduced pressure in a desiccator over calcium chloride.

3. The mixture melts below the melting point of pure acetamide, which is 81°.

4. The bomb (2 × 5.75 in. i. d.) is made from 1/4 in. seamless tubing, and the screw cap is sealed with a lead gasket. At the 1-mmole level a sodium peroxide fusion bomb (Parr[2] Model A72AC2) with 15-ml. liner is suitable.

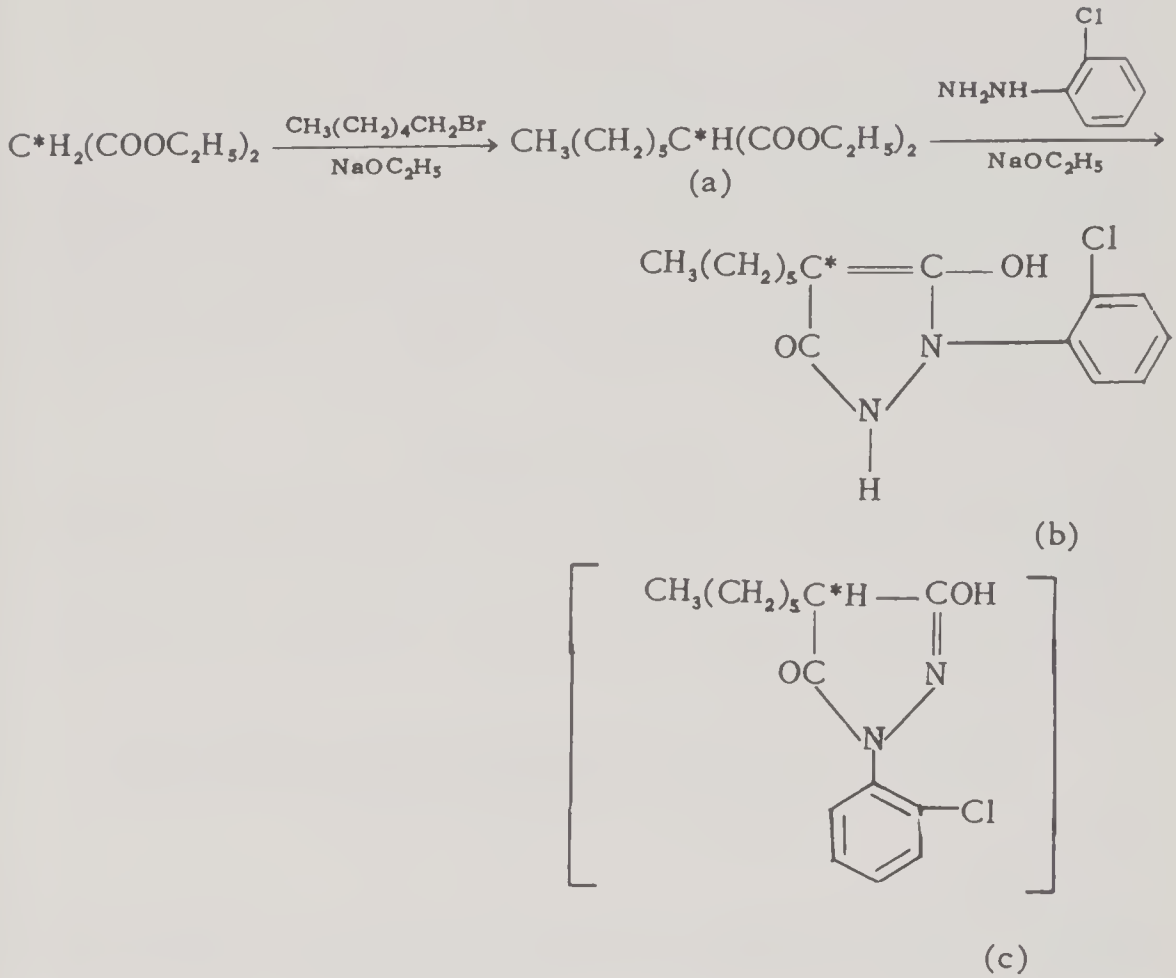
5. The product is not extracted with chloroform at pH's above the end point of phenolphthalein. The extractor is protected with a soda-lime tube, and reduction in pH due to hydrolysis of the amide is guarded against.

6. The recovery from a 1.5-mmole mock reaction mixture is 97.4%, m.p. 287–288°. The yield in trial preparations was 70–78%; 67% at the 1-mmole level.

[1]H. R. Henze, U.S. 2,409,755; Chem. Abstracts, *41*, 1250 (1947); H. R. Henze and L. M. Long, J. Am. Chem. Soc., *63*, 1936 (1941); H. R. Henze and L. G. Nunn, Jr., J. Org. Chem., *12*, 543 (1947).

[2]Parr Instrument Co., Moline, Ill.

2-(*o*-CHLOROPHENYL)-4-HEXYL-3-HYDROXY-3-PYRAZOLIN-5-ONE-4-C^{14}



P. E. Gagnon, J. L. Boivin, R. MacDonald and L. Yaffe, Can. J. Chem., 32, 823 (1954).

A. Procedure (Note 1)

(a) *Ethyl Hexylmalonate-2-C^{14}*. To a solution of 5.6 g. (0.242 mole) of sodium in 400 ml. of absolute ethanol is added 20 g. (0.125 mole) of ethyl malonate-2-C^{14}, followed by 21 g. (0.125 mole) of hexyl bromide. The mixture is refluxed until it tests acidic to litmus, then is evaporated to dryness under vacuum. The residue is treated with water, and the oil is extracted into ether solution which is dried over sodium sulfate and concentrated. The product is collected by distillation, b.p. 154–156° (10 mm.); yield 23 g. (63.3%).

(b) *2-(o-Chlorophenyl)-4-hexyl-3-hydroxy-3-pyrazolin-5-one-4-C^{14}* (Note 2). Ethyl hexylmalonate-2-C^{14} (4.0 g., 17 mmoles) is reacted overnight with 2.4 g. (17 mmoles) of *o*-chlorophenylhydrazine (Note 3) in the presence of the sodium ethoxide prepared from 2.4 g. (110 mmoles) of sodium in 200 ml. of absolute alcohol. The solvent is evaporated, and an aqueous solution of the residue is washed with ether. The product is

obtained by acidification with 33% acetic acid and recrystallized from petroleum ether; yield 62%, m.p. 154–155°.

B. Notes

1. The procedure is an adaptation of the method of Gagnon.[1]

2. Three tautomeric forms of the structure (b), rather than the classical form (c), are assigned to these compounds on the basis of infrared and ultraviolet absorption spectral studies in neutral and alkaline solutions.

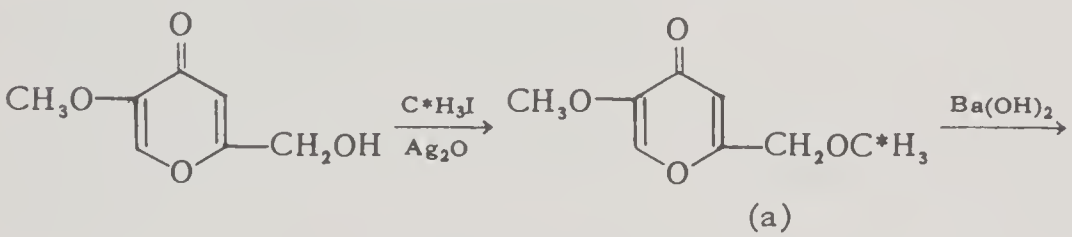
3. The aromatic hydrazines are prepared by diazotizing the corresponding anilines, and reducing with sodium sulfite. Hexylhydrazine is prepared from hexyl bromide and hydrazine in alcohol.

C. Other Preparations

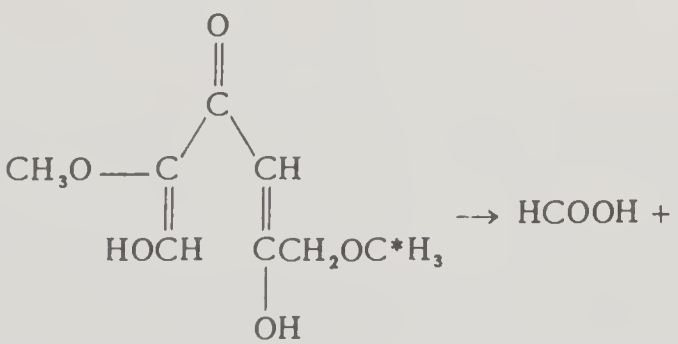
In the same manner are prepared the 2-(*m*-chlorophenyl)-, 2-(*p*-chlorophenyl)- and 2-hexyl- derivatives of 4-hexyl-3-hydroxy-3-pyrazolin-5-one-4-C^{14}.

[1]P. E. Gagnon, J. L. Boivin and P. A. Boivin, Can. J. Res., *28B*, 720 (1950).

2-(METHOXY-C^{14}-METHYL)-5-METHOXY-4*H*-PYRAN-4-ONE
(Kojic Acid C_1^{14}-Dimethyl Ether)



(a)



$$[CH_3OCH_2COCH_2COCH_2OC^*H_3] \longrightarrow CH_3COCH_2OC^*H_3 + C^*H_3OCH_2COOH$$

(d) (b) (c)

$$(b) \xrightarrow{NaOI} CHI_3 + C^*H_3OCH_2COOH$$

H. R. V. Arnstein and R. Bentley, J. Chem. Soc., *1951*, 3436.

A. Procedure (Note 1)

(a) *2-(Methoxy-C^{14}-methyl)-5-methoxy-4H-pyran-4-one* (Note 2). A mixture of 1.0 g. of 2-(hydroxymethyl)-5-methoxy-4*H*-pyran-4-one (Note 3) and 25 ml. of methanol, boiling under reflux, is treated with 4 g. of silver oxide and 5 ml. of methyl-C^{14} iodide in two portions (Note 4). The mixture is heated for 20 minutes, then filtered, and the precipitate is thoroughly washed with hot methanol. The filtrate is evaporated to dryness, the residue is washed with ether, and the crude product (0.87 g.) is recrystallized from chloroform-ether-petroleum ether. The melting point is raised from 84–85° to 88–89° by vacuum sublimation (Note 5).

(b) *Methoxy-C^{14}-2-propanone* (Note 6). A mixture of 0.25 g. of 2-(methoxy-C^{14}-methyl)-5-methoxy-4*H*-pyran-4-one, 25 ml. of water and 0.47 g. of barium hydroxide octahydrate is heated at 100° for 30 minutes, then stored for 16 hours at room temperature. The mixture is steam-distilled, 170 ml. of distillate being collected. The product may be converted, for assay purposes, to the 2,4-dinitrophenylhydrazone by treatment with the reagent in 6 *N* hydrochloric acid solution. The derivative melts at 155° after recrystallization from alcohol; the specific activity is 44.8% of that of the starting material. Degradation studies indicate that there is no isotopic rearrangement (Note 7).

(c) *Methoxy-C^{14}-acetic Acid* (Note 5). The steam distillation residue is acidified with 1.5 ml. of sulfuric acid in 10 ml. of water, the precipitate is removed by centrifuging (Note 8), and the product is isolated by extraction in the manner described (Note 7). The molar specific activity is 55.3% of that of the starting material (Note 9).

B. Notes

1. The procedure is incidental to a study of the mechanism of alkaline cleavage of γ-pyrones.

2. The procedure is that of Armit and Nolan.[1]

3. The starting material may be readily prepared[2] (72% yield) by methylating the phenolic hydroxyl group of kojic acid with methyl sulfate and alkali. Diazomethane also methylates this position.[1]

4. The second portion of silver oxide and methyl iodide is added after the vigorous reaction has subsided.

5. The methyl-C^{14} iodide was used in excess to determine the possibility of exchange with the nonisotopic 5-methoxyl group. The molar specific activity of the recovered methyl-C^{14} iodide was identical with that of the starting material, thereby excluding that possibility. The compound is assayed as *S*-methyl-C^{14}-thiuronium picrate, m.p. 224–225° after recrystallization from acetone.

The chromatography and degradation of kojic acid are described by Arnstein.[4]

6. The degradation of the γ-pyrone is by the procedure of Yabuta.[3]

The isotope dilution technique has been used by Arnstein to demonstrate the absence of any transitory pyruvic acid formation in the hydrolysis of the related compound 5-methoxy-4-oxo-4*H*-pyran-2-carboxylic acid, thus demonstrating the cleavage of *O*-methylcomenic acid exclusively at the C_2-C_3 bond. Substitution of γ-pyrones at C-2 by an electrophylic group apparently greatly increases the tendency to rupture at the C_2-C_3 bond.

7. An aliquot of the distillate is treated with excess sodium hypoiodite, and the nonisotopic iodoform is filtered off. The filtrate is acidified, and iodine is removed by extraction with a small volume of ether. The solution is continuously extracted with ether for 18 hours, then concentrated, and the methoxy-C^{14}-acetic acid is dissolved in water and converted to the silver salt, for assay, by treatment with silver carbonate. The soluble salt solution is evaporated to dryness, and the product is crystallized from dilute alcohol. The molar specific activity is the same as that of the methoxy-C^{14}-2-propanone.

8. The nonisotopic formic acid is oxidized to carbon dioxide, for assay, by heating the solution under reflux with a suspension of 3 g. of mercuric oxide.

9. Since the molar specific activity of the acid is 23% greater than that of the ketone, there is a more frequent rupture of a C_2-C_3 bond in the γ-pyrone than of a C_3-C_4 bond; since intermediate (d) is symmetrical, this must take place in part, at least, while the hydroxymethylene group is still intact.

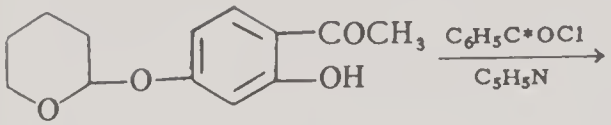
[1]J. W. Armit and T. J. Nolan, J. Chem. Soc., *1931*, 3023.

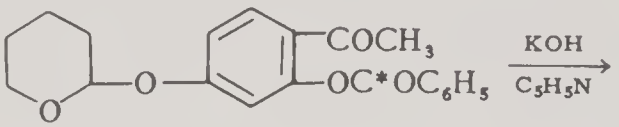
[2]K. N. Campbell, J. F. Ackerman and B. K. Campbell, J. Org. Chem., *15*, 221 (1950).

[3]T. Yabuta, J. Chem. Soc. Japan, *37*, 1185, 1234 (1916); through Chem. Abstracts, *17*, 1475 (1923); H. R. V. Arnstein and R. Bentley, Biochem. J., *54*, 493 (1953).

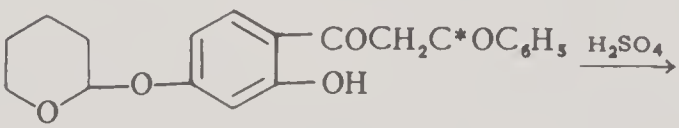
[4]H. R. V. Arnstein and R. Bentley, Biochem. J., *62*, 403 (1956); *54*, 493, 508, 517 (1954).

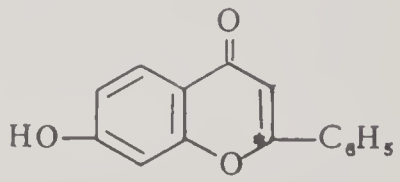
7-HYDROXYFLAVONE-2-C^{14}
(7-Hydroxy-2-phenylchromone-2-C^{14})





(a)





(b) (c)

H. Schmid and K. Banholzer, Helv. Chim. Acta, 37, 1706 (1954).

A. Procedure (Note 1)

(a) *2′-Benzoyloxy-C^{14}-4′-(tetrahydro-2-pyranyloxy)acetophenone.* To 0.777 g. of benzoyl-C^{14} chloride at 0° is added dropwise a cold solution of 1.302 g. of 2′-hydroxy-4′-(tetrahydro-2-pyranyloxy)acetophenone (Note 2) in 20 ml. of pyridine. The mixture is stored for 30 minutes at 0° and for 24 hours at 33–35°, then evaporated under vacuum. The residue is decomposed with ice water and extracted into ether solution, which is washed with 3% hydrochloric acid and saturated sodium bicarbonate. The solvent is evaporated, and the residue is distilled at 10 μ; yield 1.865 g. (99.5%), m.p. 111.5–112° after recrystallization from alcohol-ether.

(b) *1-Phenyl-3-[2-hydroxy-4-(tetrahydro-2-pyranyloxy)phenyl]-1,3-propanedione-1-C^{14}.* To a mixture of 0.925 g. of 2′-benzoyloxy-C^{14}-4′-(tetrahydro-2-pyranyloxy)acetophenone in 30 ml. of pyridine at 80° is added 1.4 g. of pulverized potassium hydroxide. The mixture is stirred vigorously for 2 hours at 80° with the exclusion of moisture (Note 3), then cooled, concentrated to 1/3–1/4 volume under reduced pressure, cooled again and acidified with 20% acetic acid (50% excess). The product is extracted into ether solution, which is washed with 3% hydrochloric acid until the wash liquor is about pH 3, then with sodium bicarbonate solution, dried and evaporated; yield 0.791 g. (85%), m.p. 98–102°. Purifica-

tion is accomplished by recrystallization from ether-petroleum ether, alcohol, and methylene chloride-petroleum ether; m.p. 104–106° (Note 4).

(c) *7-Hydroxyflavone-2-C^{14}*, (*7-Hydroxy-2-phenyl-4H-1-benzopyran-4-one-2-C^{14}*). The crude diketone is cyclized by dissolution in 15 ml. of chilled concentrated sulfuric acid and standing for 4 hours at 20°. The mixture is cooled to −10° and poured on ice, and the product is collected on a filter and washed with water; yield 64%. The melting point is 239–240°, after recrystallization from methanol and from ethyl acetate (Note 5). Purification is accomplished also by vacuum sublimation at 250° (50 μ).

B. Notes

1. These syntheses are incidental to a tracer study of the base catalyzed Baker-Venkataraman rearrangement[1] of *o*-aroyloxyacetophenones into *o*-hydroxydiaroylmethanes. The intramolecular character of the reaction was established by the failure of a mixture of 2′-mesitoyloxy-4′-methoxyacetophenone and 2′-mesitoyloxy-4′-(tetrahydro-2-pyranyloxy)-acetophenone to give cross-products. In the case of the corresponding nonsterically hindered 2′-benzoyloxy compound there results about 13–17% exchange due to transesterification.

2. Directions are given by Schmid for preparing the ether from 2′,4′-dihydroxyacetophenone and dihydropyran with a drop of polyphosphoric acid; yield 57%, m.p. 75–77°.

3. The potassium salt of the diketone precipitates.

4. A dirty brownish-green color is given with alcoholic ferric chloride.

5. 7-Acetoxyflavone-2-C^{14} is prepared by refluxing 7-hydroxyflavone-2-C^{14} with acetic anhydride for 2 hours; yield quantitative, m.p. 129–130° from ether-petroleum ether.

6. Three recrystallizations from ether-petroleum ether raise the melting point to 51–52°.

7. The melting point is raised to 101–102° by recrystallization from ether-petroleum ether, alcohol and methylene chloride-petroleum ether.

8. The extract is washed with 5% potassium hydroxide until the ether solution no longer gives a color reaction with ferric chloride. The crude product is purified by chromatography on 160 g. of neutral aluminum oxide (dried at 110°) and eluted with benzene-petroleum ether (70 : 30).

9. A wine-red color is given with alcoholic ferric chloride.

10. The ring closure is effected by heating with polyphosphoric acid for 1 hour at 90°, since concentrated sulfuric acid gives extensive decomposition. The flavone sublimes at 50 μ and 250°. Methylation in cellosolve with diazomethane gives 7-methoxy-2′,4′,6′-trimethylflavone, m.p. 137.5–138.5°.

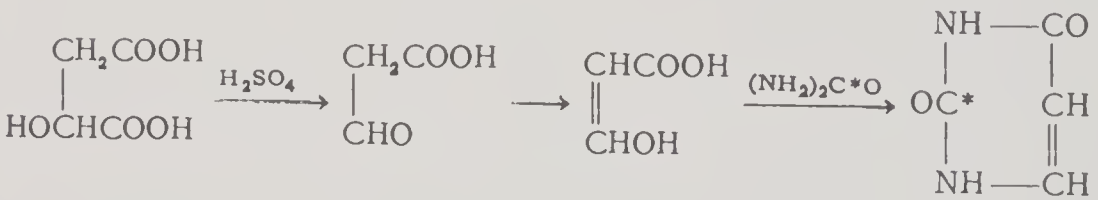
C. Other Preparations

In a similar manner from benzoyl-C^{14} chloride and 2′-hydroxy-4′-methoxyacetophenone are prepared: 2′-benzoyloxy-C^{14}-4′-methoxyacetophenone (yield 88%, m.p. 48–50°; Note 6), 1-phenyl-3-(2-hydroxy-4-methoxyphenyl)-1,3-propanedione-1-C^{14} (yield 84%, m.p. 98–100°, Notes 4 and 7) and 7-methoxyflavone-2-C^{14} (yield 78%, m.p. 111.5–112.5° from alcohol-water and ether-petroleum ether).

From 2,4,6-trimethylbenzoyl-C^{14} chloride and 2′-hydroxy-4′-(tetrahydro-2-pyranyloxy)acetophenone are prepared: 2′-(2,4,6-trimethylbenzoyloxy-C^{14})-4′-(tetrahydro-2-pyranyloxy)acetophenone (yield 84%, m.p. 71.5–74.5°; Note 8), 1-mesityl-3-[2-hydroxy-4-(tetrahydro-2-pyranyloxy) phenyl] -1,3-propanedione-1-C^{14} (yield 94%, m.p. 111.5–114° after recrystallization from ether-petroleum ether, Note 9), 7-hydroxy-2′,4′,6′-trimethylflavone-2-C^{14} (yield 70%, m.p. 282–284° from ethyl acetate and from methanol, Note 10).

[1]W. Baker, J. Chem. Soc., *1933*, 1381; *1934*, 1953; H. S. Mahal and K. Venkataraman, Current Science, *2*, 214 (1933); J. Chem. Soc., *1934*, 1767.

URACIL-2-C^{14}
[2,4(1*H*,3*H*)-Pyrimidinedione-2-C^{14}]



H. G. Mandel and C. L. Brown, J. Am. Chem. Soc., *74*, 2439 (1952).

A. Procedure

Fuming sulfuric acid (20 ml., 18% SO_3) is cooled to −5° (Dry Ice-alcohol bath) in a three-necked, 50-ml. flask fitted with a mercury sealed stirrer, thermometer and a funnel-shaped inlet tube. Finely pulverized malic acid (4.4 g., 33 mmoles) is added with stirring, while the temperature is kept below 0° (Note 1). Dry pulverized urea-C^{14} (1.7 g., 28 mmoles) is then added in small portions over a period of 10 minutes, as

the temperature is kept below 5°. The mixture is warmed slowly to 80–85° and stirred for 1 hour; then the solution is cooled and poured over 60 g. of crushed ice. The product, which separates after standing in the refrigerator for 48 hours, is centrifuged, resuspended repeatedly in water, collected and dried (Note 2); yield 1.95 g. (62%). Filter paper chromatography shows the presence of only one radioactive compound.

B. Notes

1. In the presence of concentrated sulfuric acid, malic acid eliminates the elements of formic acid and forms the transitory malonaldehydic acid,[1] which is the reactive intermediate; see uracil-4-C^{14}. Slight changes in SO_3 content have a decisive effect on the yield of the final product; the concentration used and the ratio of quantities of reagents are optimal.

2. Recrystallization from hot water is unnecessary, as purity is high.

C. Other Preparations

In a similar manner is prepared[2] uracil-5-C^{13}-4-C^{14} from urea and malic-3-C^{13}-4-C^{14} acid. The latter is formed by deamination of aspartic-3-C^{13}-4-C^{14} acid with an excess of potassium nitrite in glacial acetic acid, followed by acidification of the solution with concentrated sulfuric acid, extraction of the filtered solution with ether and concentration under reduced pressure. The over-all yield is 25% based on aspartic acid.

Bennett[3] has prepared uracil-2-C^{14} in 73% yield by desulfurizing 2-thiouracil-2-C^{14} with chloracetic acid, by the method of Wheeler and Liddle[4] (see thymine-2-C^{14}). Filter paper chromatography shows the presence of only one active compound.

The preparation of uracil-2-C^{14} by condensation of urea-C^{14} with malonate has been reported by Rutman.[5] Paper chromatography (1-butanol-water), the ultraviolet absorption spectrum, and the failure of urease to liberate carbon-C^{14} dioxide all show the absence of impurities.

[1]D. Davidson and O. Baudisch, J. Am. Chem. Soc., *48*, 2379 (1926).

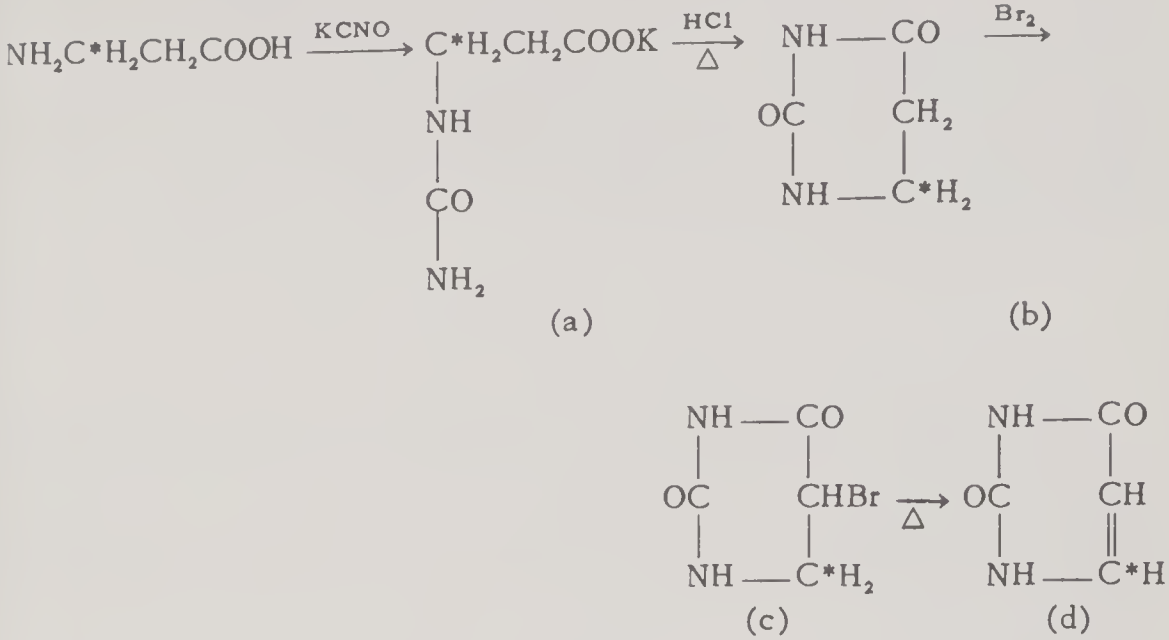
[2]U. Lagerkvist, Acta Chem. Scand., 7, 114 (1953).

[3]L. L. Bennett, Jr., J. Am. Chem. Soc., *74*, 2432 (1952).

[4]H. L. Wheeler and L. M. Liddle, Am. Chem. Jour., *40*, 547 (1908).

[5]R. J. Rutman, A. Cantarow and K. E. Paschkis, Cancer Research, *14*, 119 (1954).

URACIL-6-C^{14}



P. Fritzson, Acta Chem. Scand., 9, 1239 (1955).

A. Procedure

(a) N-*Carbamoyl-β-alanine-3-*C^{14} *Potassium Salt* (Note 1). The twice recrystallized β-alanine-3-C^{14} prepared from 0.126 g. of potassium cyanide-C^{14} and 0.244 g. of potassium chloroacetate is reacted with 0.160 g. of potassium cyanate at 25° according to the procedure of Lengfeld,[1,2] which follows (Note 2).

An aqueous solution of 2 g. of β-alanine and 1.85 g. of potassium cyanate is slowly evaporated on a water-bath. The syrupy residue crystallizes upon standing. The hygroscopic salt is stored in a desiccator; yield nearly quantitative.

(b) *Hydrouracil-6-*C^{14}, (*5,6-Dihydrouracil-6-*C^{14}) (Note 3). The crude potassium salt is acidified with 0.1 *N* hydrochloric acid, and the solution is evaporated to dryness. The residue is heated to 170°, and the crude product is crystallized from water; yield 0.119 g., 56% based on chloroacetic acid. Recrystallization from water, after decolorizing with carbon, gives a product melting at 274–275° (Note 2).

(c) *5-Bromohydrouracil-6-*C^{14}. The bromination of 0.040 g. of hydrouracil-6-C^{14} is carried out according to the method of Gabriel,[2] which follows.

A mixture of 10 g. of dry powdered hydrouracil, 40 ml. of glacial acetic acid and 14 g. of bromine is heated at 100° with shaking (Note 4)

until the bromine is decolorized (1 hour). The mixture is diluted with 40 ml. of water and stored overnight. The crystalline product is collected (12 g.) and recrystallized from 60 parts of boiling alcohol (Note 5).

(d) *Uracil-6-C^{14}* (Note 6). The crude 5-bromouracil-6-C^{14} is heated to 200°, and the crude product is crystallized from water; yield 0.0346 g., 88% based on hydrouracil. Recrystallization from water, after treatment with carbon, gives a product melting at 335° (Notes 1 and 7).

B. Notes

1. For a similar preparation, see *N*-carbamoyl-C^{14}-aspartic acid.

2. Hydrolysis[3] of hydrouracil-6-C^{14} in 0.2 *N* barium hydroxide at room temperature (about 1 hour), followed by removal of barium with 2 *N* sulfuric acid (pH 2.0) and crystallization of the product from water, gives *N*-carbamoyl-β-alanine-3-C^{14} (m.p. 169–170°) in essentially quantitative yield.

3. The procedure is that of Lengfeld[1] and Gabriel[2] (yield 83% based on β-alanine).

4. A diagram of a shaking, steam-heated tube bath is given by Gabriel.[2]

5. 5-Bromohydrouracil melts at 195° (rapid heating), decomposing quantitatively into uracil.

6. The procedure is that of Gabriel,[2] who found that the bromopyrimidine dissociates quantitatively into hydrobromic acid and uracil when heated above its melting point. Fritzson has reported unsuccessful attempts to oxidize hydrouracil with alloxan according to the quantitative method of Johnson.[4]

7. An excellent degradation of isotopically labeled pyrimidines is presented by Lagerkvist.[5] Uracil-5-C^{13}-4-C^{14} is converted through the series: hydrouracil-5-C^{13}-4-C^{14}, β-alanine-2-C^{13}-1-C^{14}, hydracrylic-2-C^{13}-1-C^{14} acid, acrylic-2-C^{13}-1-C^{14} acid, propionic-2-C^{13}-1-C^{14} acid, ethylamine-1-C^{13} (and carbon-C^{14} dioxide), acetic-1-C^{13} acid. methylamine and carbon-C^{13} dioxide. Oxidation of uracil-5-C^{13}-4-C^{14} with alkaline permanganate forms urea, which is degraded with urease to carbon dioxide.

C. Other Preparations

In a similar manner from β-alanine-1-C^{14} and -2-C^{14} are prepared uracil-4-C^{14} and -5-C^{14}, respectively.

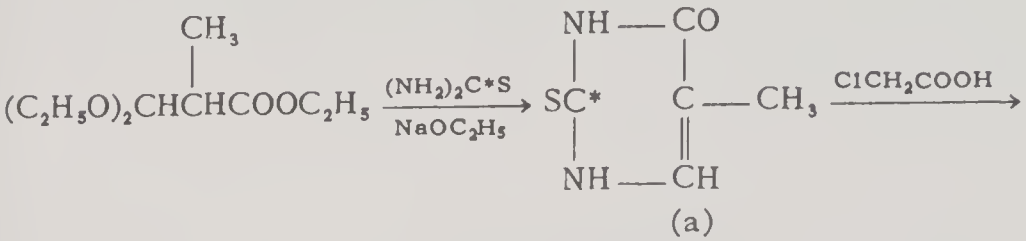
[1]F. Lengfeld and J. Stieglitz, Am. Chem. J., *15*, 516 (1893).

[2]S. Gabriel, Ber., *38*, 630 (1905).

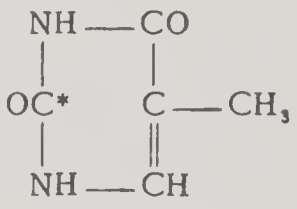
[3]R. D. Batt, J. K. Martin, J. McT. Ploeser and J. Murray, J. Am. Chem. Soc., *76*, 3663 (1954).

[4]T. B. Johnson, J. Am. Chem. Soc., 63, 263 (1941).
[5]U. Lagerkvist, Acta Chem. Scand., 7, 114 (1953).

THYMINE-2-C^{14}
(5-Methyluracil-2-C^{14})



(a)



(b)

L. L. Bennett, Jr., J. Am. Chem. Soc., 74, 2432 (1952).

A. Procedure

(a) *2-Thiothymine-2-C^{14}*. Thiourea-C^{14} is condensed with ethyl 3,3-diethoxy-2-methylpropionate (Note 1) in the presence of sodium ethoxide by the same general procedure described for the preparation of 2-thiouracil-2-C^{14}. The yield of crude product is 40–50%. The product may be recrystallized from water.

(b) *Thymine-2-C^{14}*. The desulphurization of thiothymine-2-C^{14} is effected, according to the prodedure of Wheeler and Liddle,[1] by boiling with 1.5 molecular proportions of chloroacetic acid in dilute aqueous solution for 1 hour. The solution is evaporated to dryness on a steam-bath; yield 43–55%. The product is recrystallized from water and dried over phosphorus pentoxide under vacuum. Filter paper chromatography and radioautography show the presence of only one isotopic compound. The degradation of thymine is described by Elwyn and Sprinson.[2]

B. Notes

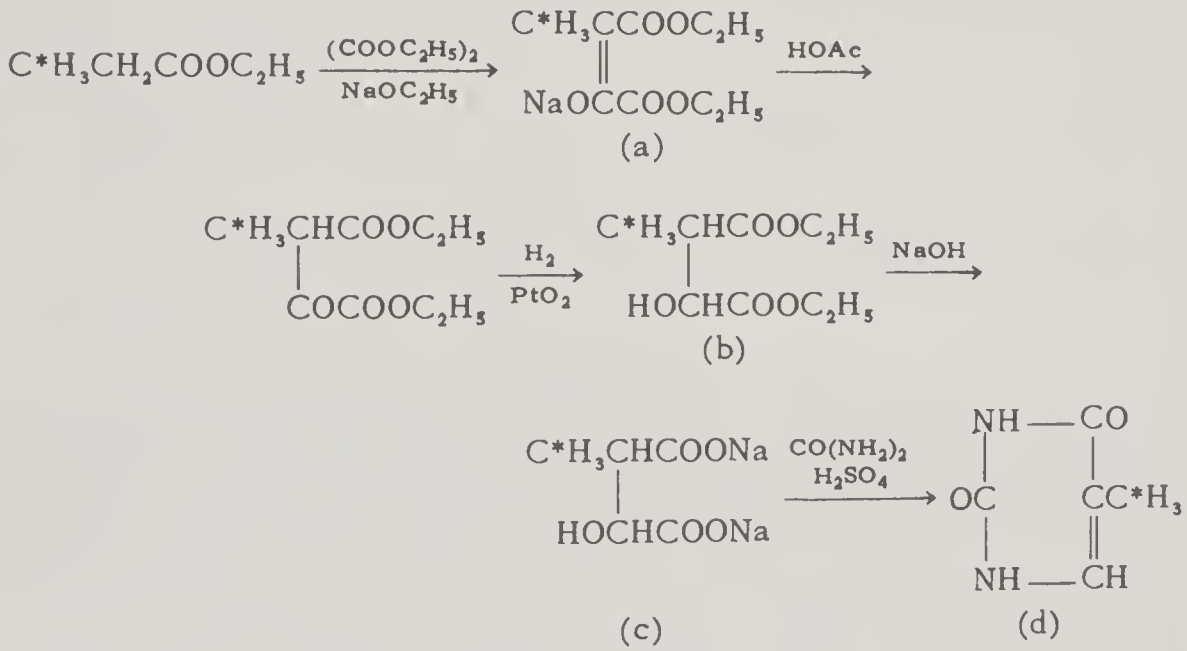
1. The starting material is prepared from ethyl 2-bromopropionate and ethyl orthoformate by a Reformatsky reaction, b.p. 96–99° (18 mm.); see 2-thiouracil-2-C^{14}, Note 3.

C. Other Preparations

The preparation of thymine-2-C^{14} by the described procedure has been reported by Holmes,[3] and the conversion (enzymatic) to thymidine-2-C^{14}, (thymine-2-C^{14} 2-deoxyriboside), is described by Friedkin.[4]

[1]H. L. Wheeler and L. M. Liddle, Am. Chem. Jour., *40*, 547 (1908).
[2]D. Elwyn and D. B. Sprinson, J. Am. Chem. Soc., *72*, 3317 (1950); J. Biol. Chem., *207*, 467 (1954).
[3]W. L. Holmes, W. H. Prusoff and A. D. Welch, J. Biol. Chem., *209*, 503 (1954).
[4]M. Friedkin and D. Roberts, J. Biol. Chem., *207*, 257 (1954).

5-METHYL-C^{14}-URACIL
(C_1^{14}-Thymine)



R. B. Henderson, R. M. Fink and K. Fink, J. Am. Chem. Soc., 77, 6381 (1955).

A. Procedure (Note 1)

(a) *Ethyl Methyl-C^{14}-sodiumoxalacetate.* (Note 2). The ethyl propionate-3-C^{14}, prepared from 0.278 mmole of sodium propionate-3-C^{14}, is vacuum-transferred into a flask containing 0.48 mmole of sodium ethoxide, 0.49 mmole of ethyl oxalate and 0.3 ml. of absolute ether. The flask is closed, and the mixture is shaken for 2 hours and then heated for a few minutes in a boiling water-bath, while being vented to the atmosphere through a trap cooled with liquid nitrogen.

(b) *Ethyl 3-Methyl-C^{14}-malate.* To the residual orange-red salt at 0° is added a mixture of 0.01–0.02 g. of Adams platinum oxide catalyst, 0.5 ml. of dilute acetic acid (0.63 meq.) and 1.5 ml. of alcohol (Note 3). The flask is cooled with liquid nitrogen, evacuated, flushed with hydrogen and filled with one atmosphere of hydrogen. The flask is sealed and shaken for 2 hours at room temperature (Note 4).

(c) *Sodium 3-Methyl-C^{14}-malate.* The flask is vented, and the contents are shaken with 1.0 ml. of 2.34 *N* sodium hydroxide at room temperature for 2 hours (Note 5), then evaporated at room temperature (50μ).

(d) *5-Methyl-C^{14}-uracil.* To 4 ml. of 20% fuming sulfuric acid, cooled with an ice-salt-bath, is added 1 g. of urea in portions, while keeping the temperature below 10°. To the residual sodium salt is added 2 ml. of this solution, and the flask is heated on a steam-bath for 2.5 hours with occasional swirling. The mixture is cooled and dissolved in water (Note 6), and the solution is adjusted to pH 4.0 by adding Amberlite IRA-400 (OH^- form) and filtered. The combined filtrate and wash liquor is concentrated and chromatographed on paper (Note 7), developed with 2-butanol saturated with water. The purified product is eluted from the paper sections with absolute alcohol; yield 0.00224 g. (6.4%, Note 8). The molar specific activity is unchanged from that of the sodium propionate-3-C^{14}.

B. Notes

1. The procedure is an adaptation of that of Scherp;[1] also see uracil-2-C^{14}. All reactions are carried out in one flask without purification of intermediates, except for the removal of volatiles. On the gram-scale the over-all yield is about 30%.

2. The procedure for the Claisen condensation is essentially that of Cox and McElvain.[2] At the gram-scale the yield is 60–70%. Also see ethyl oxalacetate-3-C^{14}.

3. Ethyl 2-ethoxalylpropionate[2] boils at 114–116° (10 mm.).

4. Paper chromatography does not reveal the presence of any ethyl methylsuccinate. In large-scale runs the yield is 85–95%, b.p. 121–124° (10 mm.). The product gives a negative color test with ferric chloride.

5. Trial experiments indicate that hydrolysis is 98–100% complete.

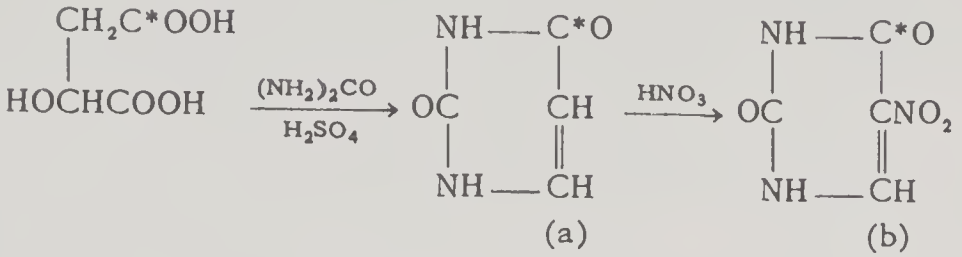
6. Ultraviolet assay indicates the presence of 0.00485 g. of product (14% yield).

7. The filter paper is put through a chromatographic wash before use.

8. Over-all yields range from 3.3 to 17.0%. At the gram-scale, the yield for the last step is 50–55%.

[1]H. M. Scherp, J. Am. Chem. Soc., 68, 912 (1946).
[2]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 272.

5-NITROURACIL-4-C^{14}



J. L. Fairley, L. L. Daus and B. Krueckel, J. Am. Chem. Soc., *75*, 3842 (1953).

Procedure

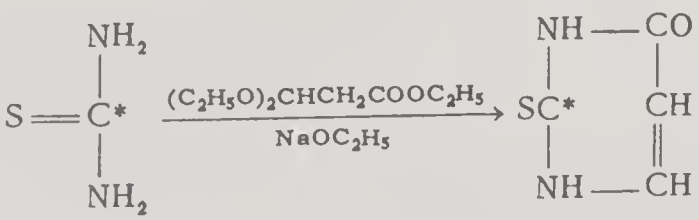
(a) *Uracil-4-C^{14}*. Urea and malic-4-C^{14} acid are caused to react in fuming sulfuric acid by the method of Davidson and Baudisch; see uracil-2-C^{14}. The product is decolorized with carbon and recrystallized from water.

(b) *5-Nitrouracil-4-C^{14}*. Nitration is effected by the method of Johnson and Matsuo,[1] which follows. The gain in weight is 94% of the theoretical; the product is recrystallized from water.

Uracil is dissolved in 4.5 parts of nitric acid (sp. gr. 1.5), and the solution is evaporated at 50–60°. The residue is washed with cold water and dried in a desiccator over sulfuric acid, giving an almost quantitative yield of quite pure product, which gives a negative test[2] for uracil. The nitration takes place only with difficulty with acid of sp. gr. 1.4.

[1]T. B. Johnson and I. Matsuo, J. Am. Chem. Soc., *41*, 782 (1919).
[2]H. L. Wheeler and T. B. Johnson, J. Biol. Chem., *3*, 183 (1907).

2-THIOURACIL-2-C^{14}



L. L. Bennett, Jr., J. Am. Chem. Soc., *74*, 2432 (1952); Nucleonics, 7 (4), 50 (1950).

A. Procedure (Note 1)

A mixture of 1.35 g. of crude thiourea-C^{14} (Note 2), 4.2 g. of ethyl 3,3-diethoxypropionate,[1] and a sodium ethoxide solution, prepared from 0.7 g. of sodium and 35 ml. of absolute alcohol, is refluxed for 4 hours. The solvent is removed in a stream of nitrogen, the residue is dissolved in cold water, and the product is precipitated by the addition of cold 50% acetic acid. The crude yield is 1.1 g. (Note 3), and the product may be recrystallized from water (Note 4).

B. Notes

1. The procedure is a modification of that of Wheeler.[2]

2. Purity 85%, m.p. 160–165°.

3. The yield is 40–55% based on barium carbonate. No difference in yield is noted when the diethyl acetal is contaminated with considerable amounts of ethyl 2-ethoxyacrylate, as occurs in its preparation[1] from ethyl bromoacetate and ethyl orthoformate. The derivative, uracil-2-C^{14}, prepared from this product shows the presence of only one active compound by paper chromatography.

4. Purification also is accomplished[3] in 5 *N* ammonium hydroxide solution by washing through a short column of Dowex-1 with warm 5 *N* ammonium hydroxide containing 1 g. of ammonium chloride per 25 ml. Paper chromatography (1-butanol-water) has disclosed only one spot visible under ultraviolet light, in agreement with an authentic sample, R_f 0.55. Scanning of the chromatogram of a high level run revealed the presence of three contaminants, or decomposition products, containing 4–6% of the total activity.

C. Other Preparations

The preparation of 2-thiouracil-2-C^{14} (12% yield) by condensation of thiourea-C^{14} and the sodium derivative of malonaldehydic ester in aqueous solution has been reported by Skipper.[4] The yield was decreased when sodium ethoxide was used in alcohol; also see 2-thiouracil-4-C^{14}.

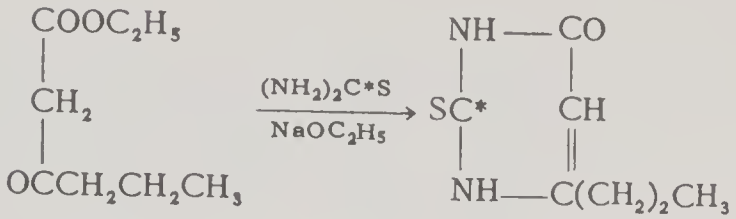
[1] N. C. Deno, J. Am. Chem. Soc., 69, 2233 (1947); E. Dyer and T. B. Johnson, J. Am. Chem. Soc., 56, 222 (1934).

[2] H. L. Wheeler and L. M. Liddle, Am. Chem. J., 40, 547 (1908).

[3] L. L. Bennett, Jr. and H. T. Baker, private communication.

[4] H. E. Skipper and L. L. Bennett, Nucleonics, 7 (4), 50 (1950).

6-PROPYL-2-THIOURACIL-2-C^{14}



A. Murray III, and A. R. Ronzio, Atomic Energy Commission Report, AECU-621; Nuc. Sci. Abstracts, *4*, 421 (1950).

A. Procedure (Note 1)

To a solution of 0.0421 g. (1.84 mmoles) of sodium in 2.75 ml. of absolute ethanol (Note 2) is added 0.175 ml. (1.05 mmoles) of ethyl 3-oxohexanoate (Note 3), followed by a solution of 0.0666 g. (0.875 mmole) of thiourea-C^{14} in 2.5 ml. of absolute alcohol. The mixture is shaken mechanically for 12 hours, while being heated under reflux at 85° (Note 4). After standing at room temperature for 5 hours, the mixture is evaporated to dryness under a gentle stream of air. The residue is dissolved in 20 ml. of 3% potassium hydroxide and continuously extracted with ether for 6 hours (Note 5). The aqueous solution is strongly acidified with sulfuric acid, forming a flocculent precipitate, which is collected by ether extraction for 3–6 hours. The extract is concentrated by evaporation, and the crude product is dried in a vacuum desiccator; yield 0.108 g. (73%). Recrystallization from boiling water (5.5 ml.) gives 0.0851 g. of colorless needles, m.p. 205–210°. Purification is accomplished by vacuum sublimation at 130–160° (10 μ); yield 0.0778 g., 52.3% (Note 6).

B. Notes

1. The procedure is adapted from the method of Anderson[1] for preparing 5- or 6-substituted 2-thiouracils from thiourea and β-ketoesters.

2. The concentration of sodium in a stock solution is determined by titration of an aliquot. The reaction mixture is protected by a guard tube filled with Ascarite and Drierite.

3. Directions are given by Anderson[1] for preparing the ester by condensing sodium ethyl acetoacetate with butyryl chloride, and then cleavingt the product in ether solution by treatment with gaseous ammonia. Six methods are described[1] for preparing β-ketoesters.

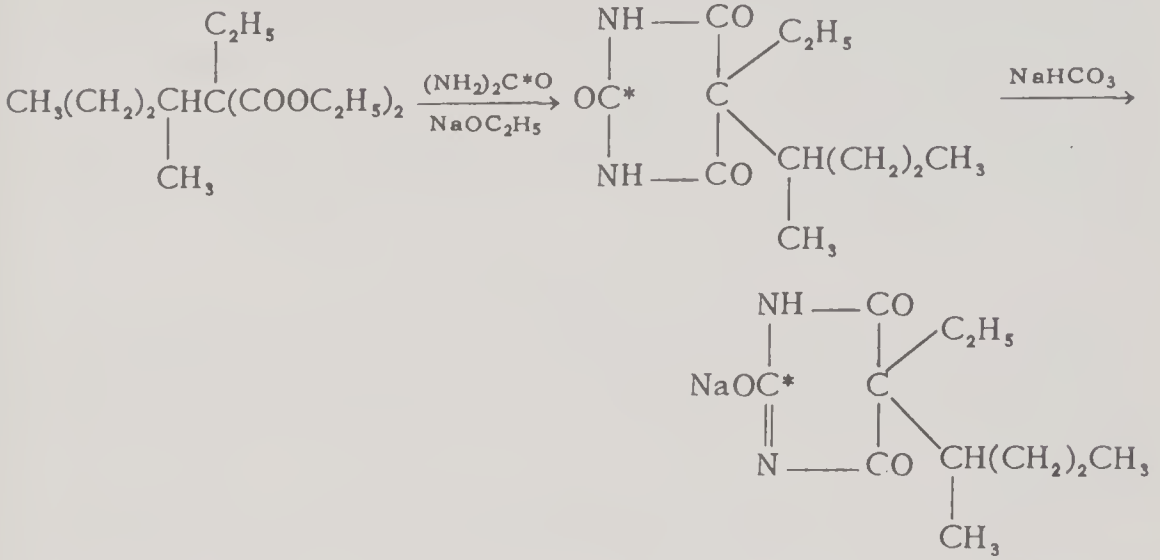
4. A colorless precipitate forms within 10 minutes.

5. The concentrated extract is mildly radioactive.

6. A purified authentic sample melts sharply at 213.5–214°.

[1]G. W. Anderson, I. F. Halverstadt, W. H. Miller and R. O. Roblin, Jr., J. Am. Chem. Soc., *67*, 2197 (1945).

SODIUM 5-ETHYL-5-(1-METHYLBUTYL)BARBITURATE-2-C^{14}
(Pentobarbital-2-C^{14} Sodium)



A. Murray III and A. R. Ronzio, Atomic Energy Commission Report, AECU-86; Nuc. Sci. Abstracts, *2*, 909 (1949).

A. Procedure (Note 1)

To a solution of 0.35 g. of sodium in 6.1 ml. of absolute ethanol (Note 2) in a 15-ml. tube is added 1.35 ml. (5.0 mmoles) of ethyl 2-ethyl-2-(1-methylbutyl)malonate, followed by 0.300 g. (5.0 mmoles) of dry urea-C^{14}. The tube is closed with a semiball joint and clamp, and placed upright in an oven adjusted to 85°. After being warmed a few minutes, the tube is shaken to completely dissolve all reactants; then the mixture is heated for 5 days. The contents are washed into a beaker with water and methanol, and the mixture is evaporated to dryness with the help of an infrared lamp and a gentle air stream. The residue is dissolved in 20 ml. of water, and the solution is made basic with 1.5 ml. of 50% potassium hydroxide solution and extracted continuously with ether for 17 hours. The clear, colorless aqueous solution is acidified strongly with hydrochloric acid (2 ml. of excess of that required to precipitate an oil) and extracted with ether for 12 hours (Note 3). The extract is evaporated, and the crude 5-ethyl-5-(1-methylbutyl)barbituric-2-C^{14} acid is dried in a vacuum desiccator over phosphorus pentoxide; yield 0.8768 g. (77.8%). Purification is achieved by decolorizing in ether solution with charcoal, evaporating the filtrate and twice recrystallizing the residue from benzene-petroleum ether (1:5) at 0°; yield 0.7441 g., m.p. 124–126°. Recrystallization from water yields glistening microneedles, m.p. 129–130° (79% recovery).

The sodium salt is formed by treating the acid with the stoichiometric amount of aqueous sodium bicarbonate solution, warming to solution and evaporating to dryness.

B. Notes

1. The procedure is adapted from the method of Dickey and Gray[1] for preparing barbituric acid.

2. Comparable yields were obtained in trial runs with potassium methoxide, potassium ethoxide or sodium methoxide.

3. When the extraction is complete, the aqueous solution no longer displays a purple fluorescence under ultraviolet excitation.

C. Other Preparations

5-Ethyl-5-(1-methylbutyl)-2-thiobarbituric-2-C^{14} acid has been prepared[2] in a similar manner from thiourea-C^{14}. The column and paper chromatography and countercurrent distribution of the compound have been described by Winters.[3]

A preparation of 5-ethyl-5-(1-methylbutyl)barbituric-2-C^{14} acid, essentially that described, is reported in detail by Titus.[4] Homogeneity was established by paper chromatography (1-butanol saturated with 1% ammonia),[5] and countercurrent distribution (chloroform and aqueous buffer of pH 9.5).

The preparation of 5,5-diethylbarbituric-2-C^{14} acid from 5 mmoles of urea-C^{14} and 10 mmoles of butyl diethylmalonate in the presence of 15 mmoles of sodium butoxide (7 hours at 100°) has been described by Goldschmidt;[7] yield 78.5%, m.p. 190°.

For a similar preparation of barbituric-2-C^{14} acid, see C_1^{14}-vitamin B_2.

[1] *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 60.

[2] D. L. Tabern, Abbott Laboratories, N. Chicago, Ill.

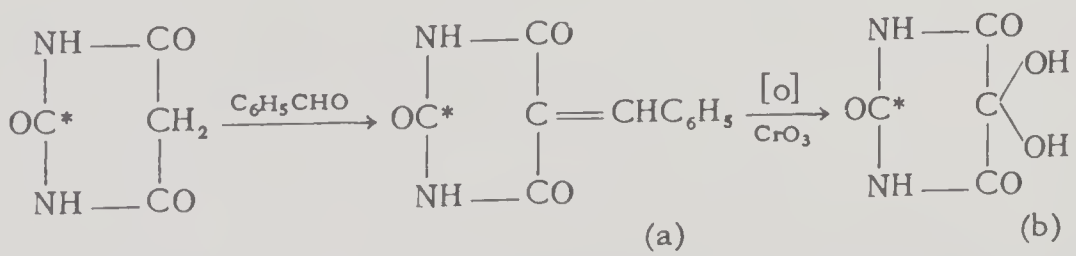
[3] W. D. Winters, E. Spector, D. P. Wallach and F. E. Shideman, J. Pharm. Exp. Therap., *114*, 343 (1955).

[4] E. Titus and H. Weiss, J. Biol. Chem., *214*, 807 (1955).

[5] L. J. Roth, E. Leifer, J. R. Hogness and W. H. Langham, J. Biol. Chem., *178*, 963 (1949).

[6] S. Goldschmidt and R. Wher, Hoppe-Seyler's, Z. physiol. Chem., *301*, 107 (1955).

ALLOXAN-2-C^{14}



(a) (b)

M. L. Tamayo, J. M. Gamboa and E. F. Alvarez, Anales real soc. espan. fés. y quím, *48B*, 889 (1952); through Chem. Abstracts, *48*, 1278 (1954).

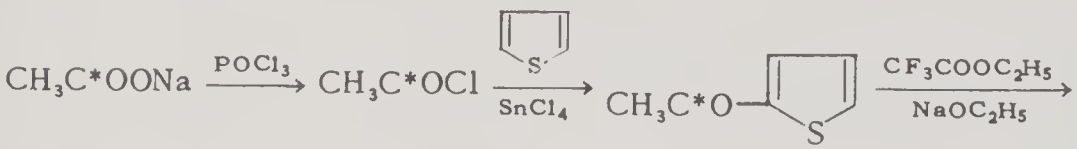
A. Procedure

(a) *5-Benzylidenebarbituric-2-C^{14} Acid.* A solution of 12.5 g. of barbituric-2-C^{14} acid in 100 ml. of water is treated with 10 ml. of benzaldehyde according to the procedure of Speer and Dabovich;[1] yield 12.5 g., m.p. 256°.

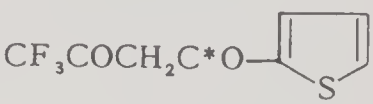
(b) *Alloxan-2-C^{14} Monohydrate,* (*2,4,5,6(1H,3H)-Pyrimidinetetrone-2-C^{14} Monohydrate*), (*Mesoxalylurea-C^{14}*). The 5-benzylidinebarbituric-2-C^{14} acid (12 g.) is oxidized with 10.9 g. of chromic anhydride in 6.5 ml. of water and 53 ml. of glacial acetic acid, according to the procedure of Speer and Dabovich.[1] The crude yield is 6.4 g.; the purified yield is 4.6 g., m.p. 255–256°. The tetrahydrate crystallized from water is converted to the monohydrate by heating at 100°, or by exposure to the air. The anhydrous product is formed by heating at 150°, or by vacuum sublimation.

[1]*Organic Syntheses*, Vol. 21, Wiley, New York, 1941, p. 5.

4,4,4-TRIFLUORO-1-(2-THIENYL)-1,3-BUTANEDIONE-1-C^{14} [2-Thenoyl-(C^{14}-trifluoroacetone)]



(a)



(b)

V. F. Raaen and G. A. Ropp, Atomic Energy Commission Report, ORNL-1288; Nuc. Sci Abstracts, 6, 4759 (1952).

A. Procedure (Note 1)

(a) *Methyl 2-Thienyl Ketone-C^{14},* (*2-Acetothienone-1-C^{14}*) (Note 2). A stirred mixture of 2.72 g. (33 mmoles) of dried sodium acetate-1-C^{14}, 10.2 g. of distilled phosphorus oxychloride and 30 ml. of dry benzene is heated at such a rate as to effect distillation through a short vigreux column over a 2-hour period. The distillate is collected in a 100-ml. receiver (Note 3) containing 4.21 g. (50 mmoles) of thiophene in 40 ml. of dry benzene. To the stirred solution at 0° is added a solution of 14.0 g. (40 mmoles) of freshly distilled stannic chloride in 15 ml. of dry benzene

over a period of 30 minutes. The mixture is stored for 12 hours at 5°, and 1 hour at room temperature; then the addition product is decomposed slowly with 1.2 *N* hydrochloric acid. The product is extracted into ether, washed with water and dried over magnesium sulfate (Note 4).

(b) *4, 4, 4-Trifluoro-1-(2-thienyl)-1, 3-butanedione-1-C*^{14}. The dry ether solution of methyl 2-thienyl ketone-C^{14} is filtered into a stirred cold solution of 4.75 g. (35 mmoles) of ethyl trifluoroacetate (Note 5) and 1.89 g. (35 mmoles) of sodium methoxide in 5 ml. of absolute ether under nitrogen. The mixture is stirred for 2 days, then evaporated under vacuum, and the residue is decomposed with 10% sulfuric acid. The mixture is extracted with ether, and the ether solution is treated with 35 ml. of *M* magnesium acetate, and the pH is adjusted to 6.5 with dilute ammonium hydroxide. The mixture is stirred thoroughly; then the supernatant solution is removed from the insoluble magnesium chelate by filter stick. The residue is decomposed with 70 ml. of 10% sulfuric acid, and the product is separated by steam distillation. The crystallized product is dried over phosphorus pentoxide; yield 4.82 g., m.p. 40–41°, 67% based on sodium acetate (Note 6).

B. Notes

1. The procedure is an adaptation of the method described by Reid and Calvin[1] for preparing β-diketones containing the trifluoromethyl group, by a Claisen condensation[2] between a methyl ketone and ethyl trifluoroacetate.

2. The procedure is an adaptation of that of Johnson and May.[3]

3. A diagram of the receiver is presented by Raaen. The flask is equipped with a magnetic stirring bar and a stopcock, positioned such that the flask may be used as a separatory funnel by tipping.

4. The yield of 2-acetothienone is 79–83% at the 0.2-mole scale, b.p. 89–91° (9 mm.), n_D^{20} 1.566. The semicarbazone melts at 186–197° (cor.).

5. The ester may be prepared by the method of Reid[4] or Henne.[2]

6. At times in trial runs, the product crystallized in a second form (m.p. 75–80°), which is converted to the lower-melting form by heating above 80°. According to Reid and Calvin,[1] the corrected melting points are: 2-thenoyltrifluoroacetone (42.5–43.2°), dioxime (134.3–134.7°) and the green copper chelate (242–243°). As a result of the strong negative inductive effect of the trifluoromethyl group, the ketone is sufficiently acidic to be assayed by titration.[1]

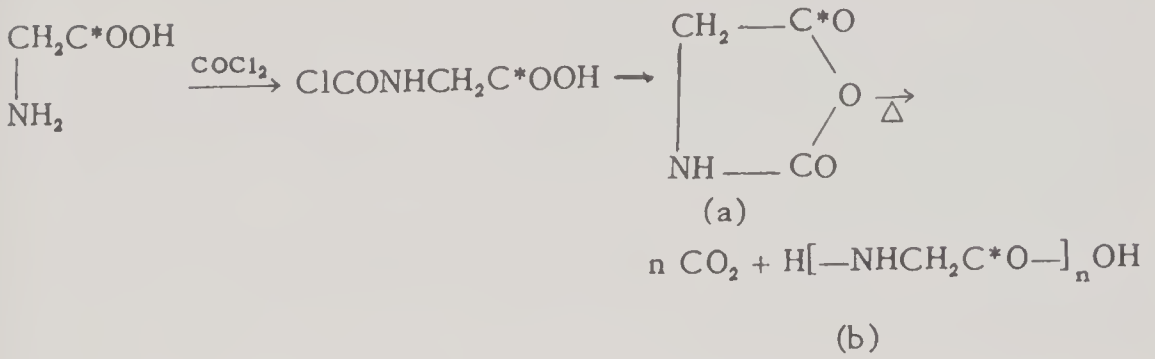
[1] J. C. Reid and M. Calvin, J. Am. Chem. Soc., *72*, 2948 (1950).

[2] A. L. Henne, M. S. Newman, L. L. Quill and R. A. Staniforth, J. Am. Chem. Soc., *69*, 1819 (1947); *Organic Reactions*, Vol. I, Wiley, New York, 1942, p. 266.

[3] *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 8.

[4] J. C. Reid, J. Am. Chem. Soc., *69*, 2069 (1947).

2,5-OXAZOLIDINEDIONE-5-C^{13}



(a)

$$n\ CO_2 + H[-NHCH_2C^*O-]_nOH$$

(b)

K. Heyns and R. Brockmann, Z. Naturforsch., *9b*, 21 (1954).

A. Procedure (Note 1)

(a) *2, 5-Oxazolidinedione-5-C^{13}*. The preparation is made in 74% yield by suitable adaptation of the procedure of Farthing,[1] which follows (Note 2).

Into a stirred suspension of 8.0 g. of powdered glycine and 375 ml. of anhydrous dioxane at 40° is slowly passed phosgene for 4–5 hours, the glycine dissolving slowly. Dry air is blown through the system overnight at room temperature (Note 3); then the solvent is removed under reduced pressure at 40°. The residual liquid solidifies under vacuum at 40°, and the product is washed out with dry ether and immediately dried under vacuum at room temperature over phosphorus pentoxide and flaked petroleum wax (Note 4).

(b) *C^{13}-Polyglycine*. 2, 5-Oxazolidinedione-5-C^{13} (0.090–0.100 g.) is dried for 1–2 hours at 30° (10 μ), then pyrolyzed by heating with an oil-bath at 112 ± 2° for 2 hours (Note 5). The carbon dioxide evolved is nonisotopic; yield 80% (Note 6).

B. Notes

1. The preparation is incidental to a study of the mechanism of polypeptide formation by the method of Leuchs.[2]

2. As the product is extremely water-sensitive, all apparatus is oven-dried for at least 1 hour at 110° and protected with Drierite.

3. The intermediate, *N*-chloroformylglycine[-1-C^{13}], may be obtained by concentrating the solution under vacuum at lower temperature. To a portion in chloroform solution was added dry aniline; the precipitated 5-phenylhydantoic[-1-C^{13}] acid was collected and recrystallized from alcohol, m.p. 195°.[1]

4. The product is stored over phosphorus pentoxide. A convenient qualitative test for 2, 5-oxazolidinediones is to cover a trace with water (faint effervescence) and add a drop of aniline (violent effervescence);

the finely divided precipitate of polypeptide is confirmed by the biuret reaction.

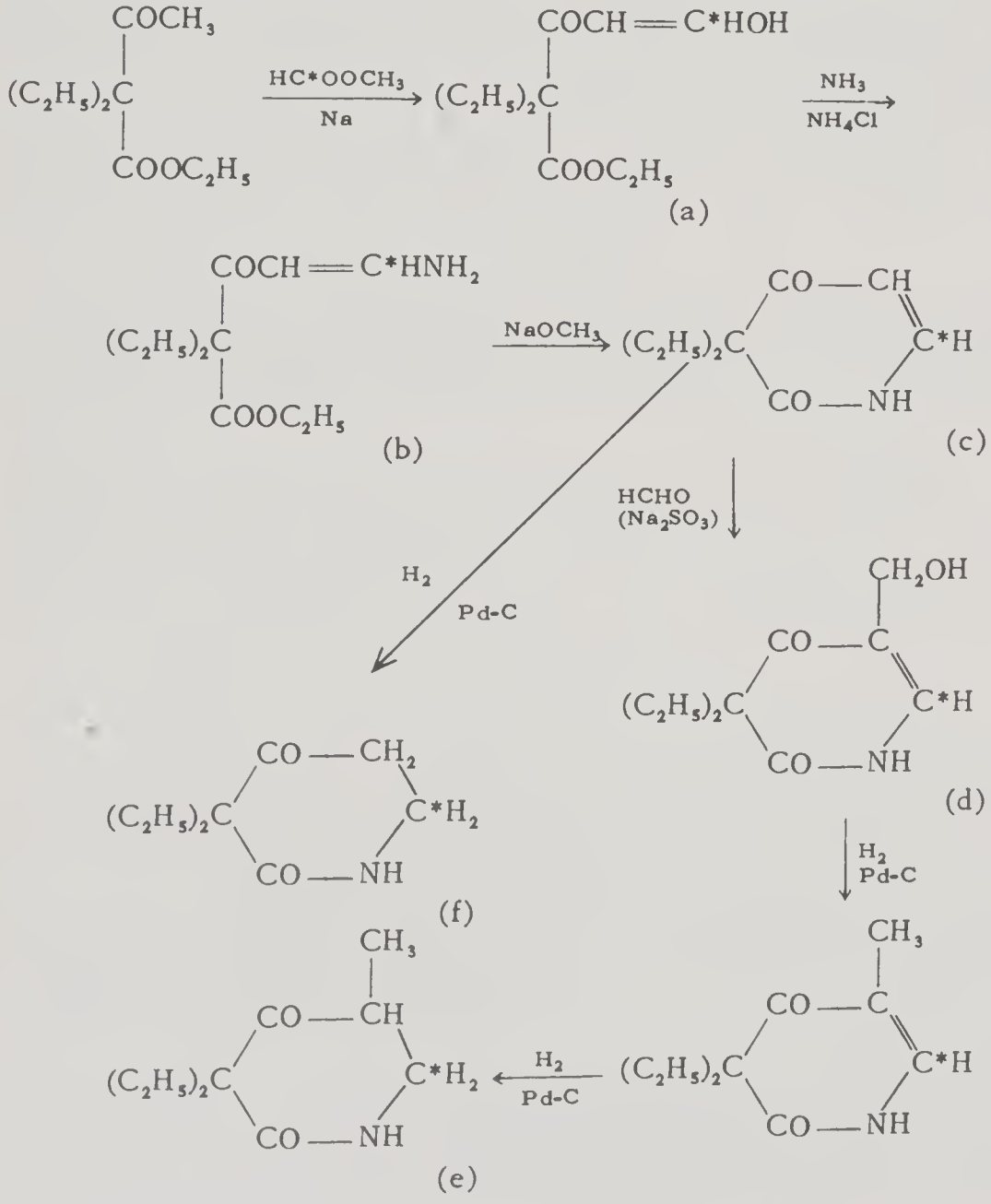
5. A diagram of the vacuum apparatus is presented by Heyns.

6. Experiment shows that the carbon dioxide is evolved with an isotope effect, indicating that this is probably the rate-determining step of the reaction mechanism.

[1]A. C. Farthing, J. Chem. Soc., *1950*, 3213; A. C. Farthing and R. J. W. Reynolds, Nature, *165*, 647 (1950); F. Fuchs, Ber., *55*, 2943 (1922).

[2]H. Leuchs, Ber., *39*, 857 (1906); R. B. Woodward and C. H. Schramm, J. Am. Chem. Soc., *69*, 1551 (1947).

3,3-DIETHYL-5-METHYL-2,4-PIPERIDINEDIONE-6-C[14]



K. Bernhard, G. Brubacher and A. H. Lutz, Helv. Chim. Acta, 37, 1839 (1954).

A. Procedure (Note 1)

(a) *Ethyl 2, 2-Diethyl-5-hydroxy-3-oxo-4-pentenoate-5-C*^{14}. To a suspension of 0.352 g. of sodium methoxide and 3.600 g. of ethyl 2, 2-diethylacetoacetate in 22.5 ml. of absolute ether at -5° under nitrogen is added, in 8 ml. of rinse ether, the methyl formate-C^{14} prepared from 0.600 g. (8.8 mmoles) of sodium formate-C^{14}. The mixture is slowly warmed to room temperature and stirred for 16 hours.

(b) *Ethyl 5-Amino-2, 2-diethyl-3-oxo-4-pentenoate-5-C*^{14}. A stirred mixture of the crude ethyl 2, 2-diethyl-5-hydroxy-3-oxo-4-pentenoate-5-C^{14} and a solution of 1.500 g. of ammonium chloride and 0.75 ml. of concentrated ammonium hydroxide in 20 ml. of water is heated at 75° for 1.5 hours. The product is extracted into three 15-ml. portions of benzene, and the solvent is evaporated.

(c) *3, 3-Diethyl-2, 4(1*H, *3*H)*-pyridinedione-6-C*^{14}. To the crude ethyl 5-amino-2, 2-diethyl-3-oxo-4-pentenoate-5-C^{14} is added a solution of 0.352 g. of sodium methoxide in 15 ml. of methanol. The mixture is heated and concentrated under reduced pressure. Unreacted ethyl 2, 2-diethylacetoacetate is removed by dilution with 30 ml. of water and extraction with 60 ml. of ether. The extract is washed with three 7.5-ml. portions of 0.01 *N* sodium hydroxide, and the combined aqueous solution is neutralized with concentrated hydrochloric acid to precipitate the product, which is extracted into 25 ml. of benzene-ether (1:1). The extract is dried and evaporated under vacuum at 50°. The semicrystalline residue is dissolved in 3 ml. of hot benzene, and the solution is treated with petroleum ether (5 ml.) to the point of incipient turbidity. The product is collected and recrystallized; yield 0.795 g. (54% based on sodium formate), m.p. $90–91^\circ$.

(d) *3, 3-Diethyl-5-(hydroxymethyl)-2, 4(1*H, *3*H)*-pyridinedione-6-C*^{14}. To a stirred suspension of 0.200 g. of 3, 3-diethyl-2, 4(1H, 3H)-pyridinedione-6-C^{14} in 0.6 ml. of water at 20° under nitrogen is added 0.036 g. of paraformaldehyde and 0.100 g. of sodium sulfite (Note 2). The mixture is stirred overnight, and the product is separated by extraction with five 5-ml. portions of ethyl acetate. The crude product, left by evaporation of the solvent at 50° under vacuum, is recrystallized from ethyl acetate-petroleum ether; yield 80%, m.p. $136–137^\circ$.

(e) *3, 3-Diethyl-5-methyl-2, 4-piperidinedione-6-C*^{14}. A mixture of 0.170 g. of 3, 3-diethyl-5-(hydroxymethyl)-2, 4(1H, 3H)-pyridinedione-6-C^{14}, 10 ml. of methanol and 0.200 g. of 5% palladium-charcoal is shaken in the presence of hydrogen at room temperature for 20 hours (Note 3). The catalyst is separated and washed with two 10-ml. portions of hot benzene. The combined filtered solution is evaporated under vacuum, and the residual oil is treated with 5 ml. of petroleum ether to induce crystallization; yield 0.103 g. (65%), m.p. $72–73^\circ$.

(f) *3, 3-Diethyl-2, 4-piperidinedione-6-C*^{14}. A mixture of 0.130 g. of crude 3, 3-diethyl-2, 4(1*H*, 3*H*)-pyridinedione-6-C^{14}, 10 ml. of methanol and 0.100 g. of 5% palladium-charcoal is shaken in the presence of hydrogen for 20 hours. The catalyst is separated and washed with benzene, and the combined solution is evaporated under vacuum. The residue is recrystallized from 3 ml. of boiling benzene, following the dropwise addition of 5 ml. of petroleum ether; yield 0.095 g., m.p. 101–102°.

B. Notes

1. The procedure is an adaptation of the method outlined by Schnider.[1]

2. The reaction takes place also in the presence of other sulfites, but not sodium bisulfite or alkali.

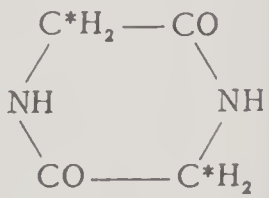
3. The hydroxymethyl group is hydrogenated almost quantitatively[1] before the ring is attacked, forming the intermediate 3, 3-diethyl-5-methyl-2, 4(1*H*, 3*H*)-pyridinedione (-6-C^{14}).

[1]O. Schnider, H. Frick and A. H. Lutz, Experientia, *10*, 135 (1954); O. Schnider, Festschrift Emil Barrell, *1936*, 195.

2, 5-PIPERAZINEDIONE-3, 6-C_2^{14}

$$NH_2C^*H_2COOH \xrightarrow[HCl]{C_2H_5OH} HCl \cdot NH_2C^*H_2COOC_2H_5 \xrightarrow[CHCl_3]{NH_3}$$

(a)



(b)

F. Kögl and A. M. Municio, Hoppe-Seyler's Z. physiol. Chem., *300*, 6 (1955).

A. Procedure

(a) *Glycine-2-C*^{14} *Ethyl Ester Hydrochloride.* Anhydrous hydrogen chloride is passed into a suspension of 10.8 g. of glycine-2-C^{14} in 82 ml. of absolute alcohol until the crystals go into solution. The product is crystallized by storage of the solution in a refrigerator; yield 17.2 g. (Note 1).

(b) *2, 5-Piperazinedione-3, 6-*C_2^{14} (Note 2). Into a stirred mixture of the ester hydrochloride and anhydrous chloroform is passed ammonia, dried over potassium hydroxide. The filtered solution is evaporated under vacuum, and the residue (12.5 g.) is mixed with 7.5 ml. of ice water and

stored for 3 days at room temperature. The crystalline product is collected and dried under vacuum over concentrated sulfuric acid; yield 4.3 g., 61% (Note 3).

B. Notes

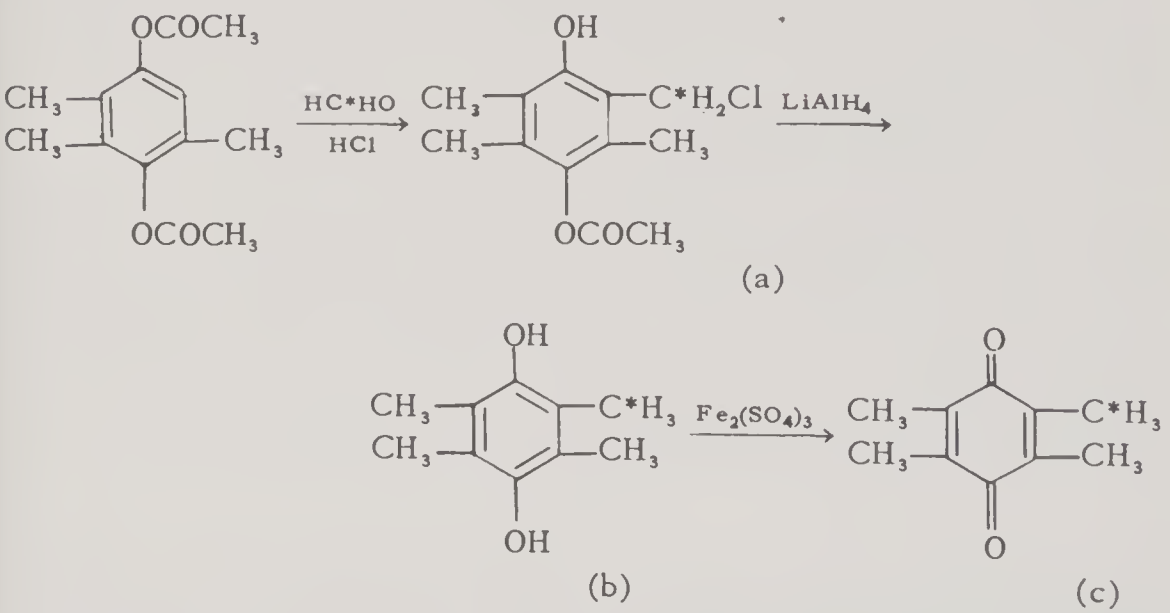
1. Glycine ethyl ester hydrochloride sublimes, m.p. 144°.

2. The ring closure is effected by the method of Viscontini.[1]

3. No reaction is observed with ninhydrin or bromphenol blue. 2, 5-Piperazinedione melts at 311–312° (dec.), sublimes at 260°, is sparingly soluble in water, and forms a hydrochloride (m.p. 129–130°).

[1]M. Viscontini, Compt. rend., *221*, 445 (1945).

TETRAMETHYL-C_1^{14}-*p*-BENZOQUINONE
(Duroquinone-α-C^{14})



A. A. Bothner-By, J. Am. Chem. Soc., 73, 4228 (1951).

A. Procedure

(a) *2-(Chloromethyl-C^{14})-3, 5, 6-trimethylhydroquinone 4-Acetate.* The yield of crude product is 1.85 g. from 2.00 g. of trimethylhydroquinone diacetate and 6.1 ml. of formaldehyde-C^{14} solution by the procedure of Smith and Carlin,[1] which follows.

A solution of 60 ml. of 40% aqueous formaldehyde and 80 ml. of hydrochloric acid is saturated at 10–20° with hydrogen chloride; the temperature is raised to 30° and maintained at that point while 20 g. of trimethylhydroquinone diacetate is added (Note 1). The mixture is stirred vigor-

ously for 1 hour while a brisk stream of hydrogen chloride is passed in. There is a sudden deposition of a semi-solid mass, which gradually disintegrates, and the colorless mixture becomes pink, red and finally purple (Note 2). The end of the reaction is usually indicated by the appearance of froth. The mixture is then poured onto 100 g. of ice, and the solid is collected, washed with cold water until the washings are colorless, and dried in a vacuum desiccator over potassium hydroxide for 10 hours. The product is refluxed with 300 ml. of ether and filtered, and the filtrate is stirred for 1 hour with 3 g. of Norit. The filtered solution is concentrated to 75 ml., diluted with an equal volume of petroleum ether (b.p. 77–115°) and cooled to crystallize the product; yield 17.8 g. (89%), m.p. 147–148° (Note 3).

(b) *Tetramethyl-C_1^{14}-hydroquinone, (Durohydroquinone-α-C^{14}).* A solution of 1.82 g. of crude chloromethyl compound in 50 ml. of absolute ether is added slowly to a stirred solution of 2.0 g. of lithium aluminum hydride in 100 ml. of ether. After an additional 30 minutes of stirring, 100 ml. of 10% hydrochloric acid is added carefully, sufficient ether is added to dissolve the product, and the extract is separated, dried and concentrated to the point of crystallization. An equal volume of petroleum ether is added, and the mixture is cooled in a refrigerator; yield 1.10 g. (89%), m.p. 220–223° (Note 5).

(c) *Tetramethyl-C_1^{14}-*p*-benzoquinone, (Duroquinone-α-C^{14}).* To a mixture of the evaporated mother liquors from (b), the aqueous layer from (b), and 0.50 g. of tetramethyl-C_1^{14}-hydroquinone is added 10 g. of ferric sulfate, and the mixture is steam-distilled (Note 4). The product is extracted from the distillate into ether, and the solvent is evaporated through a column; yield of crude product 0.50 g., m.p. 107–110°. Recrystallization from methanol gives 0.43 g., m.p. 110–111° (Note 5).

B. Notes

1. Temperature must be closely controlled throughout this reaction or the nature of the product changes greatly. The starting material dissolves in about 10 minutes.

2. Outside cooling may be required to maintain a temperature of 30°.

3. The product is sufficiently pure for most purposes. Recrystallization raises the melting point to 150–151°.

4. The system is protected with cold traps.

5. Directions are given by Bothner-By for preparing quinhydrone complexes. Experiment indicates that there is rapid exchange of isotope between the quinone and hydroquinone in solution, but none in the solid complex over a 24-hour period. Recovery of the components is achieved through thermal decomposition and sublimation. These findings disprove the formulation of quinhydrones as resonance hybrids.

As methanol solutions of durohydroquinone exposed to air are quickly autoxidized, forming duroquinone, use of a vacuum apparatus for handling the reduced solutions has been described with the aid of a diagram.[2] A kinetic study of the electron exchange reaction has been described.[2]

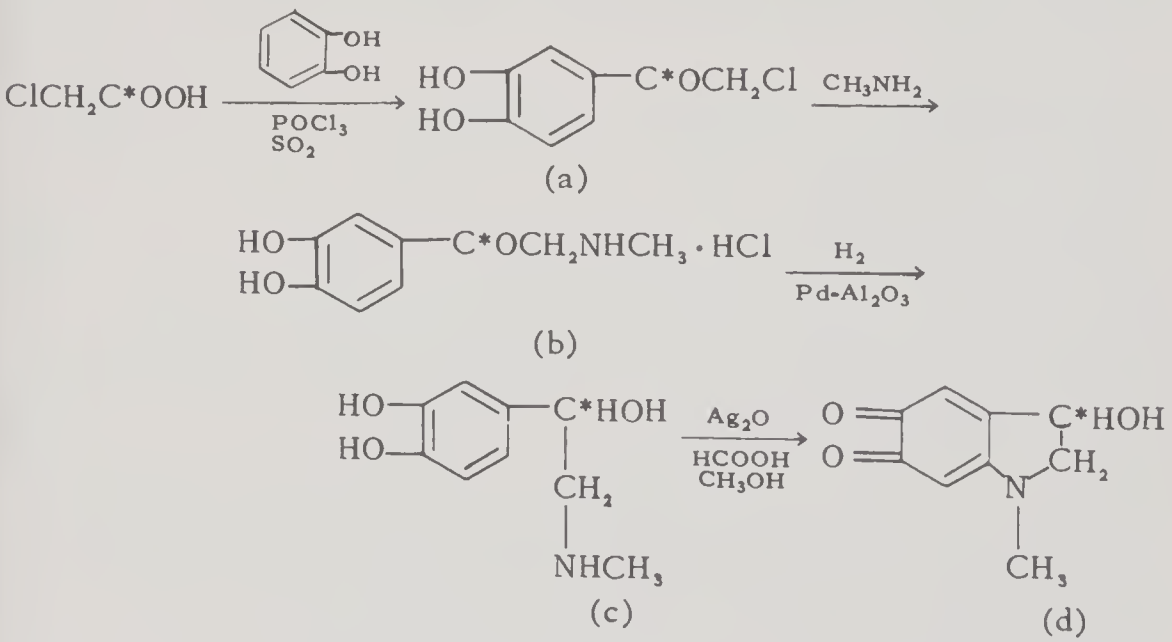
C. Other Preparations

Durohydroquinone-α-C^{14} has been prepared[2] from duraquinone-α-C^{14} by the action of sodium borohydride. To a stirred solution of 1 g. of sodium borohydride in 30 ml. of ethanol is added 1.00 g. of duroquinone-αC^{14} in 10 ml. of methanol. The colorless solution is diluted with 40 ml. of water containing 1 drop of hydrochloric acid, and chilled. The precipitated product is filtered, dried and sublimed at 170° and 50 μ; yield 93%, m.p. 242–243°.

[1]L. I. Smith and R. B. Carlin, J. Am. Chem. Soc., *64*, 524 (1942); L. I. Smith, H. E. Ungnade, J. W. Opie, W. W. Prichard, R. B. Carlin and E. W. Kaiser, J. Org. Chem. *4*, 323 (1939).

[2]A. A. Bothner-By, J. Am. Chem. Soc., *75*, 728 (1953).

3-HYDROXY-1-METHYL-5,6-INDOLINEDIONE-3-C^{14}
(C_1^{14}-Adrenochrome)



R. W. Schayer, J. Am. Chem. Soc., *74*, 2441 (1952); J. Biol. Chem., *189*, 301 (1951).

A. Procedure (Note 1)

(a) *2-Chloro-3′,4′-dihydroxyacetophenone-1-*C^{14} (Note 2). A mixture of 1.95 g. of chloroacetic-1-C^{14} acid, 1.95 g. of pyrocatechol and 2.0 ml. of freshly distilled phosphorus oxychloride is refluxed for about 45 minutes

in a stream of sulfur dioxide (Note 2). The mixture is dissolved in 30 ml. of hot water and filtered. The crude product (1.50 g.), m.p. 169–170°, is recrystallized from hot water containing traces of hydrochloric acid and sodium bisulfite; yield 1.13 g. (29%), m.p. 173°.

(b) *3′,4′-Dihydroxy-2-methylaminoacetophenone-1-C^{14} Hydrochloride, (C_1^{14}-Adrenalone Hydrochloride)*. A mixture of 1.00 g. of 2-chloro-3′, 4′-dihydroxyacetophenone-1-C^{14} and 5.0 ml. of 24% methylamine is stored at room temperature for 20 hours with frequent shaking. The mixture is diluted with 9 ml. of alcohol and stored in the cold for 1.5 hours. The brown precipitate is collected and washed in turn with 50% alcohol, absolute alcohol and ether. The crude amine is dissolved in a minimal volume of dilute hydrochloric acid, diluted to 20 ml. with water, and reprecipitated by addition of ammonia. The hydrochloride is prepared by dissolving the amine (0.52 g., 54% yield) in 3 *N* hydrochloric acid and precipitating with absolute alcohol and ether; yield 0.50 g., 81% based on adrenalone (Note 3).

(c) *3,4-Dihydroxy-α-(methylaminomethyl)benzyl-α-C^{14} Alcohol, (Adrenaline-β-C^{14})* (Note 4). A mixture of 0.24 g. of the adrenalone hydrochloride, 10 ml.of water and 0.20 g. of 5% palladium-on-aluminum oxide is hydrogenated for two hours at ordinary temperature and pressure. The catalyst is filtered off, and excess ammonia is added; yield 0.150 g. (74%) (Note 5). Purity is confirmed by paper chromatography in butanol-acetic acid, R_f 0.45 (Note 6).

L-Adrenaline-β-C^{14} is prepared[1] in 6% yield (Note 7) by resolution of DL-adrenaline-β-C^{14}, according to the method of Flächer,[2] as L-adrenaline-β-C^{14} D-bitartrate which melts at 147.9–148.5° after four recrystallizations from methanol.

(d) *3-Hydroxy-1-methyl-5,6-indolinedione-3-C^{14}* (Note 8). The adrenaline-β-C^{14} (0.100 g.) is dissolved in 3.0 ml. of absolute methanol containing 0.06 ml. of 99% formic acid. After the solution is warmed to 35°, 0.7 g. of silver oxide is added. The mixture is shaken and maintained at 35° for exactly one minute (Note 9), then quickly filtered, the residue being washed with 1 ml. of methanol. The filtrate is stored at −15° for 30 minutes, and the crystallized product is collected and washed with 1:1 methanol-ether, 1:3 methanol-ether and ether; yield 0.022 g. of reddish-brown crystals. Addition of ether to the mother liquor crystallizes a second crop; 0.018 g., 40% total yield (Note 10).

B. Notes

1. The procedure is an adaptation of Hoberman's[3] modification of the method of Stolz.[4]

2. The atmosphere of sulfur dioxide raises the yield by repressing the oxidation of pyrocatechol, while the use of freshly distilled phosphorus oxychloride reduces tar formation.

3. The chromatography of adrenalone is described by Van Espen.[5]

4. The procedure is adapted from that of Sergievskaya.[6]

5. The over-all yield is 4.4% based on barium carbonate.

6. The product has the same biological effect on the blood pressure of a dog as does an authentic sample.

7. A purity of 98% is indicated by $[\alpha]_D^{25}$ −51.0°.

8. The procedure is an adaptation of that of MacCarthy.[7]

9. Prolonged oxidation leads to the formation of melanin. According to Veer[1] the compound (m.p. *ca.* 125–130° dec.) is highly autoxidizable, and filtration in a hydrogen atmosphere is recommended.

10. Biological tests indicate that the product is free of adrenaline. Sobotka[8] states that the reddish-violet crystals keep indefinitely in the dry state at refrigerator temperature.

C. Other Preparations

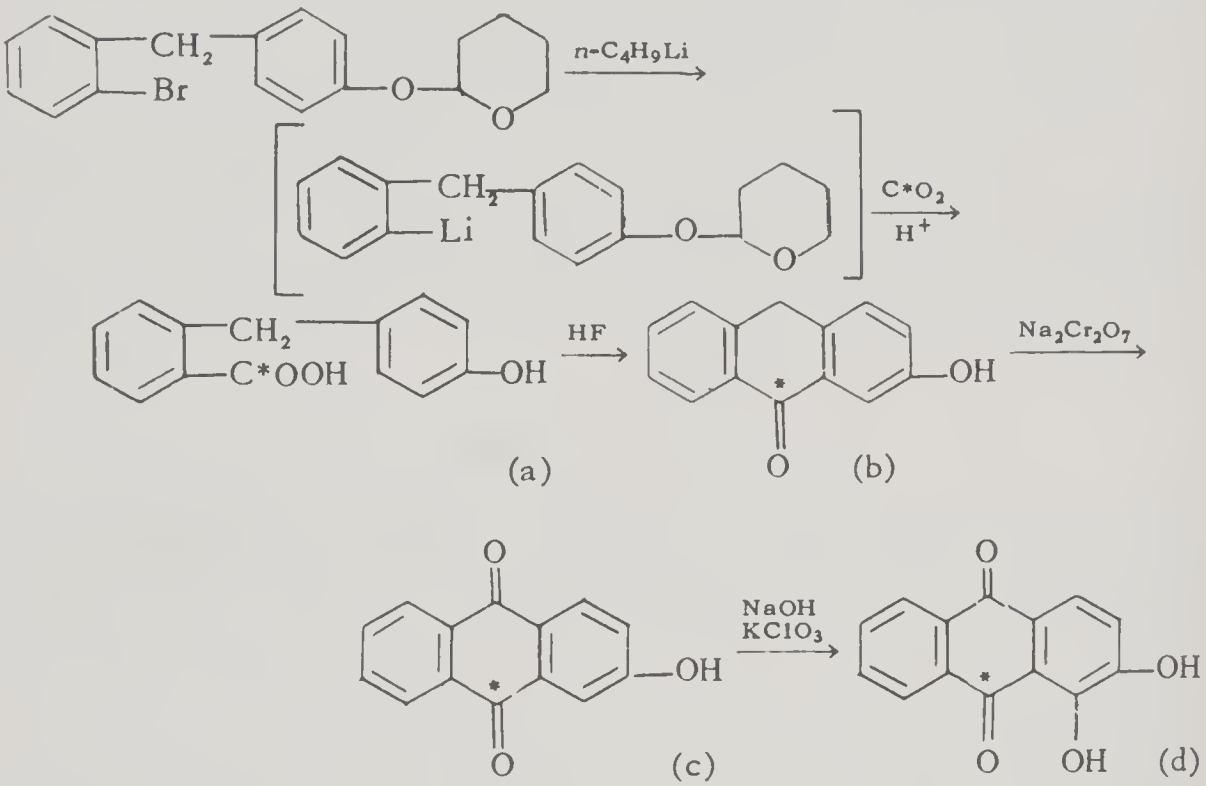
By the same procedure from chloroacetic-2-C^{14} acid are prepared:[9] 2-chloro-3′,4′-dihydroxyacetophenone-2-C^{14}, 3′,4′-dihydroxy-2-methylaminoacetophenone-2-C^{14} (and hydrochloride) and adrenaline-α-C^{14} or 3,4-dihydroxy-α-(methylaminomethyl-C^{14})benzyl alcohol (a degradation is described by Gurin).[10]

From methylamine-C^{14} are prepared:[11] 3′,4′-dihydroxy-2-methylamino-C^{14}-acetophenone (and hydrochloride), and DL- and L-3,4-dihydroxy-α-(methylamino-C^{14}-methyl)benzyl alcohol. The C_1^{14}-adrenalone was prepared in a closed system from methylamine-C^{14} hydrochloride (3 parts) in water (4 parts), by distilling aqueous methylamine-C^{14} into a flask containing 2-chloro-3′,4′-dihydroxyacetophenone (2 parts) and allowing the mixture to stand under nitrogen for 20 hours. Methylamine-C^{14} hydrochloride was recovered, and the entire process was repeated several times.

[1]W. L. Veer, Rec. trav. chim., *61*, 638 (1942).
[2]F. Flächer, Hoppe-Seyler's Z. physiol. Chem., *58*, 189 (1908).
[3]H. D. Hoberman, J. Am. Chem. Soc., *57*, 1382 (1935).
[4]F. Stolz, Ber., *37*, 4199 (1904).
[5]J. Van Espen, Mededel. Vlaam. Chem. Ver., *15*, 66 (1953); through Chem. Abstracts, *47*, 11661 (1953).
[6]S. Sergievskaya and M. Fedatova, Org. Chem. Ind. (U.S.S.R.), *2*, 96 (1936); Chem. Abstracts, *31*, 670 (1937).
[7]C. L. MacCarthy, Chim. et Ind., *55*, 435 (1946); through Chem. Abstracts, *40*, 7180 (1946); J. Harley-Mason, J. Chem. Soc., *1950*, 1276.
[8]H. Sobotka and J. Austin, J. Am. Chem. Soc., *73*, 3077 (1951).
[9]R. W. Schayer and R. L. Smiley, J. Biol. Chem., *202*, 425 (1955).

[10]S. Gurin and A. M. Delluva, J. Biol. Chem., *170*, 545 (1947).
[11]R. W. Schayer, J. Biol. Chem., *192*, 875 (1951); R. W. Schayer, R. L. Smiley and E. H. Kaplan, J. Biol. Chem., *198*, 545 (1952).

1,2-DIHYDROXYANTHRAQUINONE-9-C^{14}
(C_1^{14}-Alizarin)



D. L. Williams and A. R. Ronzio, J. Org. Chem., *18*, 489 (1953).

A. Procedure

(a) α-(p-*Hydroxyphenyl*)-*o-toluic*-C^{14} *Acid* (Note 1). To 5.5 ml. of a stirred, 0.56 *M* ethereal solution of butyllithium (3.09 mmoles, Note 2) maintained at −25° is added dropwise, during 15 minutes, a solution of 1.425 g. (4.12 mmoles) of α-(*o*-bromophenyl)-*p*-tolyl 2-tetrahydropyranyl ether (Note 3) in 10 ml. of absolute ether. The mixture is stirred at −25° for 50 minutes (Note 4), then frozen with liquid nitrogen, and the system is evacuated. The mixture is thawed at −55° to evolve trapped nitrogen, then refrozen, and the system again is evacuated at 0.1 μ, before being isolated from the pump. The frozen carbon-C^{14} dioxide from 0.4065 g. (2.06 mmoles) of barium carbonate-C^{14} is vaporized from a reservoir in contact with the thawed and rapidly stirred aryllithium solution at a bath temperature of −60°. The last of the carbon-C^{14} dioxide is distilled into the reaction flask by freezing with liquid nitrogen. The mixture is stirred

at −60° for 3 minutes, then acidified with 4 ml. of 15% hydrochloric acid, stirred for 15 minutes and stored for several hours at room temperature (Note 5). The mixture is made basic with saturated sodium bicarbonate solution and continuously extracted with ether for 24 hours. The aqueous solution is acidified with 6 *N* hydrochloric acid, and the product is extracted into ether for 46 hours. The extract is dried over magnesium sulfate, filtered and concentrated on a water-bath. The residue is brought to constant weight under vacuum, the volatile impurities being collected in a cold trap. The yield is 0.438 g. (93.3% based on barium carbonate), m.p. 132–134° (Note 6).

(b) *2-Hydroxy-9-anthrone-9-*C^{14} (Note 7). The crude carboxylic acid is transferred into a 100-ml. platinum crucible with absolute ether and evaporated to dryness. The crucible is placed in a stainless steel cylinder (2 3/4 × 3 in.), which is then closed with a transparent "Bakelite" cover, which is fitted with a stainless steel inlet tube connected to a condenser coil cooled with Dry Ice, and with an exhaust tube protected from moisture with anhydrous potassium fluoride. The cylinder is cooled with a bath at −10°, and approximately 5 ml. of anhydrous hydrogen fluoride is collected in the crucible (7 minutes). The bath temperature is allowed to rise to 20° during 30 minutes; then the inlet tube is removed, and the hydrogen fluoride is evaporated with a stream of nitrogen. The residue is washed by trituration with 5% sodium bicarbonate solution, collected on a filter, and dried. Dissolution in acetone, filtration, and evaporation of the solvent under vacuum give 0.4078 g. (100%) of a pale-yellow crude product.

(c) *2-Hydroxyanthraquinone-9-*C^{14} (Note 8). To a stirred suspension of the crude 2-hydroxy-9-anthrone-9-C^{14} in 12 ml. of acetic acid is added 0.578 g. (1 molar equivalent) of sodium dichromate in 1 ml. of hot water. The mixture is immersed in a bath at 55°, and the temperature is raised to 82° during 20 minutes and maintained there for 10 minutes. The mixture is cooled to room temperature, diluted with 30 ml. of water and treated with 14.3 g. of sodium bicarbonate to neutralize about 90% of the acid. The product is collected on a filter, washed with three portions of cold water, and air-dried. A small amount of additional product is obtained by continuous ether extraction of the mother liquors for 50 hours. A solution of the combined crude product in hot acetone is filtered and evaporated to dryness; yield 0.4342 g. (99.7%), m.p. 298–306°.

(d) *1,2-Dihydroxyanthraquinone-9-*C^{14} (Note 9). To a stirred mixture of the crude 2-hydroxyanthraquinone-9-C^{14} and 0.1 g. of powdered sodium hydroxide (Note 10), in an 8-ml. Parr bomb, is added a solution of 0.286 g. of potassium chlorate (1.2 molar equivalents) in 3 ml. of water. The stirring rod is rinsed with 1 ml. of water; then 4 g. of powdered sodium hydroxide is added to the solution, and the sealed bomb is heated for 43

hours in an oil-bath at 165–175°. The mixture is dissolved in 150 ml. of water at 60°, acidified with 6 *N* hydrochloric acid and set aside to cool. The orange precipitate is collected and washed with several portions of cold water. An additional small amount of product is obtained by continuous ether extraction (48 hours) of the combined mother liquor and washings. The purified product is collected by 6 to 12-hour periods of sublimation at 125° at 0.1–0.2 mm; yield 0.3142 g. (65.4% based on barium carbonate), m.p. 287–288°. The molar specific activity is the same as that of the starting material.

B. Notes

1. A diagram of the apparatus, consisting of a Grignard carbonation apparatus filled with dry nitrogen, is presented by Williams; also see benzoic-C^{14} acid.

2. For preparation of the reagent see *p*-aminobenzoic-C^{14} acid, Note 4.

3. Directions are given by Williams for preparing the starting material. *o*-Bromobenzoyl chloride, prepared from *o*-bromobenzoic acid with thionyl chloride, is esterified with phenol in the presence of sodium hydroxide. The phenyl *o*-bromobenzoate is converted to 2-bromo-4-hydroxybenzophenone by the Fires rearrangement, in the presence of aluminum chloride. Reduction of this compound, by the Clemmensen method, gives α-(*o*-bromophenyl)-*p*-cresol. The hydroxyl group is blocked by forming the cyclic acetal with dihydropyran, in almost quantitative yield. This excellent method of Parham and Anderson[1] is a useful means of protecting both aromatic and aliphatic hydroxyl groups in reactions in basic media.

4. The acid yield drops when longer reaction times are used, and is only 45% after 30 minutes of stirring.

5. Both the lithium compounds and the acetal are hydrolyzed by acid solution.

6. The purified acid melts at 153–154°.

7. The procedure is adapted from the method of ring closure described by Fieser and Hershberg.[2] When the ring closure is effected with concentrated sulfuric acid, the yield is 50–75% of very crude product. The purified compound melts at 221°.

8. The procedure is adapted from the method of Blicke and Weinkauff.[3]

9. The procedure is a modification of the method described by Vogel.[4]

10. The alkali is sufficient to form the sodium salt.

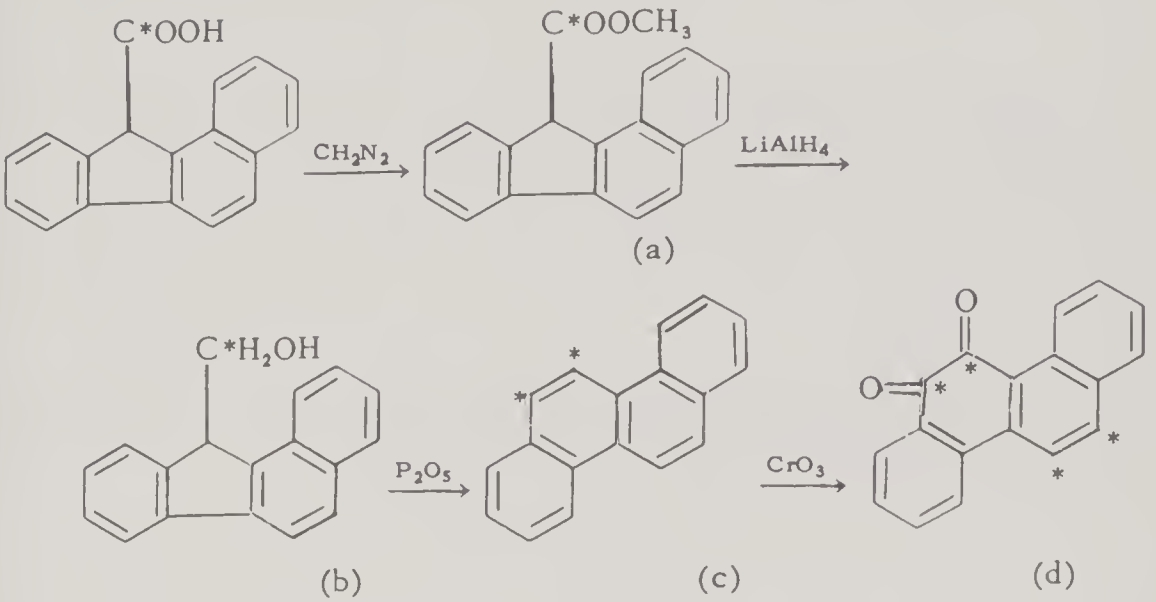
[1]W. E. Parham and E. L. Anderson, J. Am. Chem. Soc., *70*, 4187 (1948).

[2]L. F. Fieser and E. B. Hershberg, *ibid.*, *61*, 1272 (1939).

[3]F. F. Blicke and O. J. Weinkauff, *ibid.*, *54*, *1460 (1932).*

[4]A. I. Vogel, *Practical Organic Chemistry*, Longmans, Green and Co., Inc., New York, 1948, p. 850.

5,6-CHRYSENEDIONE-5,6,11,12-$C^{14}_{1/4}$



C. J. Collins, D. N. Hess, R. H. Mayor, G. M. Toffel and A. R. Jones, J. Am. Chem. Soc., 75, 397 (1953).

A. Procedure

(a) *Methyl 11-Chrysofluorenecarboxylate-C^{14}.* To a cooled solution of 0.146 g. of 11-chrysofluorenecarboxylic-C^{14} acid in 5 ml. of dry methanol is added an excess of diazomethane in ether. After 20 minutes the mixture is evaporated. An ether solution of the residue is washed with water and evaporated, giving 0.153 g. of product, which then is recrystallized from methanol; yield 73%, m.p. 133–135° (first crop).

(b) *11-Chrysofluorenemethanol-α-C^{14}.* Lithium aluminum hydride (0.5 g.) in ether is added to a solution of 0.174 g. of the ester in 5 ml. of absolute ether. After 1 hour, the excess reagent is decomposed with moist ether, and the ether solution is washed with hydrochloric acid and evaporated to dryness, yielding 0.180 g. of a yellow oil (Note 1).

(c) *Chrysene-5,6-$C^{14}_{1/2}$.* A mixture of 0.090 g. of 11-chrysofluorenemethanol-α-C^{14}, 20 ml. of dried xylene and 0.30 g. of phosphorus pentoxide is refluxed for 30 minutes. The solvent is evaporated in an air stream, an excess of sodium bicarbonate solution is added to the residue, and continuous ether extraction yields 0.079 g. of crude product. Crystallization from a benzene solution, which has been passed through a column of alumina, gives 0.043 g. of product (65%), m.p. 252–254° (Note 2). Degradation studies indicate that 76% of the activity is at C-5 (Note 3).

(d) *5,6-Chrysenedione-5,6,11,12-$C^{14}_{1/4}$.* Chrysene-5,6-$C^{14}_{1/2}$ (14.44 g.) is oxidized to the quinone by the method of Graebe and Honigsberger[1], using 62 g. of sodium dichromate and 140 ml. of glacial acetic acid; yield 11.54

g., m.p. 228–240°. After one crystallization from acetic acid, the yield is 8.92 g., m.p. 238–240°.

B. Notes

1. Attempts to crystallize this material have failed, and decomposition accompanies distillation. The *p*-nitrobenzoate melts at 167–167.5°.

2. A high level activity preparation from carbon-C^{14} dioxide, carried out without purification of intermediates, gives a 93% over-all yield of crude chrysene-5,6-$C^{14}_{1}/_{2}$. The molar specific activity is unchanged from that of the starting material.

3. A substituted 1-naphthyl position (11a) is competing for the carbinol with a substituted phenyl position (10a), during the Wagner rearrangement. For the degradation, see 11-chrysofluorenone-5,6,11-$C^{14}_{1}/_{3}$.

[1]C. Graebe and F. Honigsberger, Ann., *311*, 257 (1900).

ETHERS

ETHYL ETHYL-1-C^{14} ETHER

$$CH_3C^*H_2I \xrightarrow{NaOC_2H_5} CH_3C^*H_2OCH_2CH_3$$

J. C. Burtle and W. N. Turek, J. Am. Chem. Soc., 76, 2498 (1954).

A. Procedure (Note 1)

The apparatus consists of a 500-ml. 3-necked flask equipped with a thermometer, a dropping funnel and a small center-tube fractionating column (Note 2) fitted with a distilling head cooled with a Dry Ice-acetone cold finger. To a solution of 6.0 g. of sodium in 100 ml. of absolute ethanol, at 70–75°, is added slowly 26.4 g. ethyl-1-C^{14} iodide at such a rate as to maintain a reaction temperature above 60°. The mixture is refluxed for 15 minutes and slowly distilled, the product being collected in a receiver at −78°; yield 11.5 g. (92%), b.p. 33.5–34°.

B. Notes

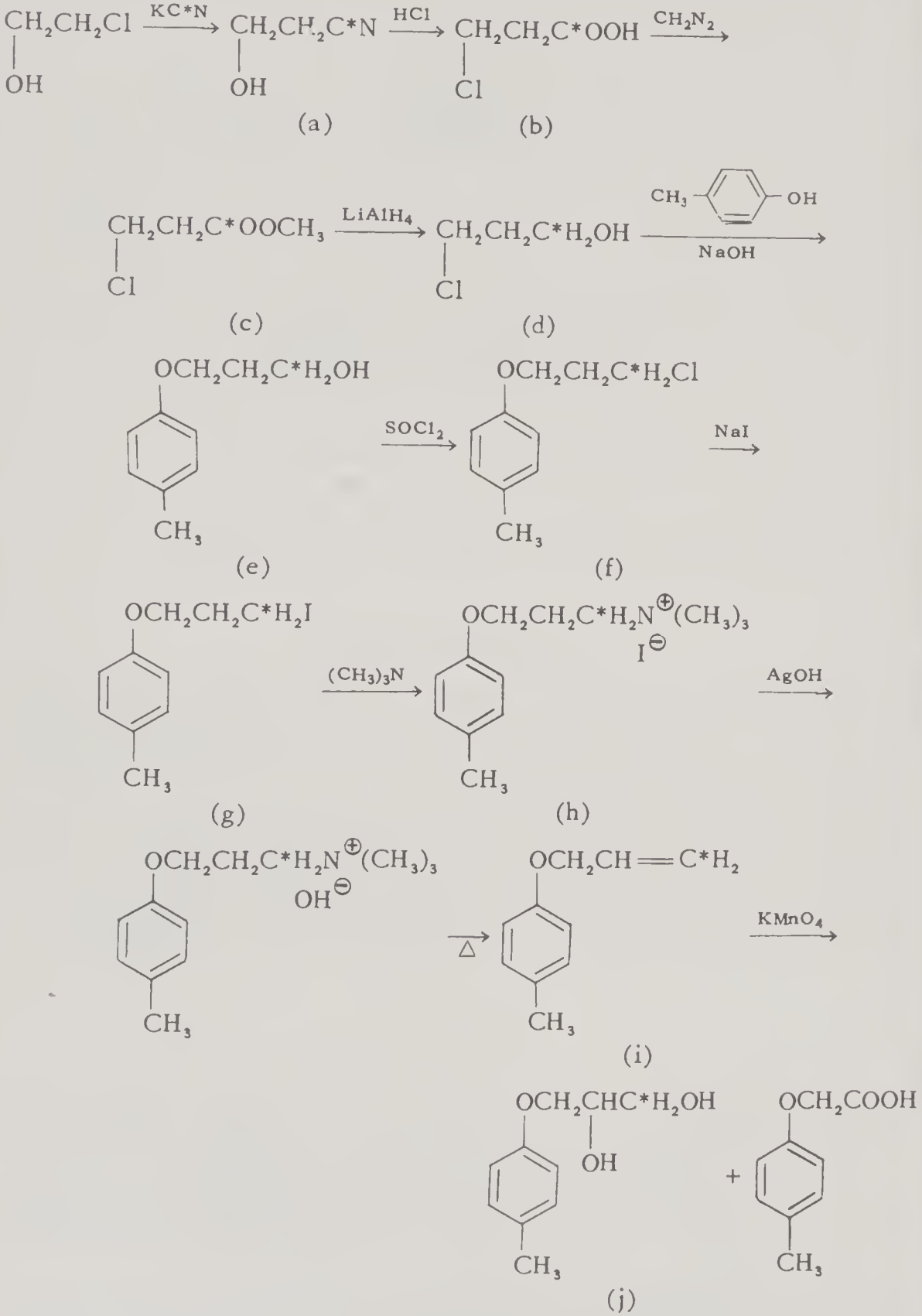
1. The procedure is an adaptation of the Williamson synthesis as modified by Bishop.[1]

2. An efficient column of this type having an H.E.T.P. of less than 4 mm. and equipped with an automatic take-off valve is described by Naragon and Lewis.[2]

[1]W. B. S. Bishop, J. Soc. Chem. Ind., *43*, 23T (1924); A. W. Williamson, J. Chem. Soc., *4*, 229 (1852).

[2]E. A. Naragon and C. J. Lewis, Ind. Eng. Chem., Anal. Ed., *18*, 448 (1946).

ALLYL-3-C^{14} *p*-TOLYL ETHER



H. Schmid and K. Schmid, Helv. Chim. Acta, 35, 1879 (1952).

A. Procedure

(a) *Hydracrylonitrile-1-C^{14}.* To a boiling solution of 10.26 g. of freshly distilled ethylene chlorohydrin in 50 ml. of absolute alcohol, under reflux, is added dropwise a solution of 8.259 g. of potassium cyanide-C^{14} in 12.6 ml. of water. The mixture is heated for 20 hours and then cooled, diluted with acetone and filtered. The filtrate is evaporated under reduced pressure, and the residue is distilled; yield 7.43 g. (83%), b.p. 109–111° (10–11 mm.).

(b) *3-Chloropropionic-1-C^{14} Acid.* The nitrile (7.43 g.) is heated, in two portions, with 25 ml. of concentrated hydrochloric acid for 3 hours at 100° in a sealed tube. Sufficient water is added to dissolve the ammonium chloride, and the mixture is extracted with several portions of ether. The extract is dried over sodium sulfate and evaporated, and the residue is sublimed at 40–60° at 0.01 mm., giving quite pure product. The aqueous solution is extracted exhaustively (overnight) with ether. The extract is combined with the sublimation residue and evaporated, and this residue again is treated with hydrochloric acid in the manner described. The combined yield is 7.94 g. (71%), m.p. 38–40°.

(c) *Methyl 3-Chloropropionate-1-C^{14}.* To a dry ether solution of 7.94 g. of 3-chloropropionic-1-C^{14} acid at 0° is added dropwise a freshly prepared ethereal diazomethane solution until the yellow color persists. After 1.5 hours, the ether is distilled through a column, and the residue is dried over sulfuric acid for 48 hours at atmospheric pressure. The yield is nearly quantitative.

(d) *3-Chloro-1-propanol-1-C^{14}.* The methyl 3-chloropropionate-1-C^{14} is diluted with an equal volume of absolute ether and, with vigorous stirring under anhydrous conditions at 0°, is treated dropwise with a 10% excess of ethereal lithium aluminum hydride (1.537 g.) solution (Note 1). The mixture is stirred for 2 hours at 0°, then cooled to −15° and hydrolyzed by the addition of moist ether, followed by dilute hydrochloric acid. The mixture is saturated with sodium chloride and exhaustively extracted with ether. The extract is dried, the solvent is distilled through a column and the product is fractionated; yield 5.323 g. (78%), b.p. 60–61° (10–11 mm.).

(e) *3-p-Tolyloxy-1-propanol-1-C^{14}.* A mixture of 1.88 g. of 3-chloro-1-propanol-1-C^{14}, 2.84 g. of freshly distilled *p*-cresol and 1.05 g. of sodium hydroxide in 11 ml. of water is heated for 3 hours at 125°. The mixture is extracted into ether, which is then washed with 10% sodium hydroxide solution and water, dried and evaporated. The yield of crystalline product is 2.784 g., 85% (Note 2).

(f) *3-Chloropropyl-3-C^{14} p-Tolyl Ether.* To the ice-cooled 3-*p*-tolyloxy-1-propanol-1-C^{14} is added dropwise 4 g. of purified thionyl chloride,

followed by three drops of anhydrous pyridine. The mixture is heated under reflux for 4 hours at 80° under anhydrous conditions, then poured into ice water and extracted with ether. The ether solution is washed with dilute hydrochloric acid and with sodium bicarbonate solution, then dried and evaporated. The residual oil is used without purification (Note 3).

(g) *3-Iodopropyl-3-C^{14}* p-*Tolyl Ether.* A mixture of the 3-chloropropyl-3-C^{14} *p*-tolyl ether, 40 ml. of anhydrous acetone and 6 g. of sodium iodide (dried under high vacuum) is refluxed for 16 hours under anhydrous conditions. The solvent is vacuum-distilled at 30° through a column. The residue is extracted with four portions of hot absolute ether, and the combined filtrates are evaporated (Note 4).

(h) *Trimethyl(3-*p*-tolyloxypropyl-1-C^{14})ammonium Iodide.* A solution of the 3-iodopropyl-3-C^{14} *p*-tolyl ether and 2.5 g. of anhydrous trimethylamine in absolute alcohol is heated for 6 hours at 100° in a sealed tube. The product is crystallized from hot alcohol; yield 4.451 g., m.p. 172–175.5° (80% based on e). The mother liquor yields a second crop (0.490 g.), after decolorization with charcoal, making the total yield 88–90% (Note 5).

(i) *Allyl-3-C^{14}* p-*Tolyl Ether.* A mixture of 4.884 g. of the quaternary iodide, 100 ml. of water and an excess of silver oxide, freshly prepared from 9 g. of silver nitrate, is vigorously stirred for 10 hours at 20°. After standing for some time, the supernatant liquid is drawn off and the precipitate is washed with hot water. The filtrate is evaporated, in small portions, under reduced pressure below 40° to a viscous brown syrup. The combined residues of trimethyl(3-*p*-tolyloxypropyl-1-C^{14})ammonium hydroxide are freed from remaining water by warming to 70° under vacuum; at about 90° decomposition begins with the distillation of water, trimethylamine and allyl *p*-tolyl ether. The pyrolysis is completed at 140° without leaving a residue (Note 6). The distillate is extracted into ether solution and washed with 2 *N* hydrochloric acid, 10% potassium hydroxide solution and water. The solvent is removed by distillation through a column, and the residue is distilled at 10.5 mm. with an air-bath temperature of 100–110°. The distillate is dissolved in pentane, then passed through a column of activated alumina (20 × 300 mm.) and evaporated; yield 1.800 g. (83.5%), n_D^{20} 1.5199. Degradation studies, see below, indicate that there is no isotopic rearrangement (Note 7).

(j) *3-*p*-Tolyloxy-1,2-propanediol-1-C^{14}.* To a vigorously stirred mixture of 0.273 g. of the olefin and 20 ml. of water at 0° is added dropwise, over a period of 1 hour, a solution of 0.975 g. of potassium permanganate in 100 ml. of water. The mixture is further stirred for 30 minutes, then decolorized with sodium bisulfite solution, acidified with dilute sulfuric acid and exhaustively extracted with ether. The ether

solution is extracted with three portions of sodium bicarbonate solution, which are then washed with fresh ether. The combined ether solutions, are dried and evaporated; yield 0.126 g. (37%). The colorless platelets melt at 71–72.5° after several recrystallizations from ether-petroleum ether; the molar specific activity is the same as that of the starting material (Note 8).

The sodium bicarbonate solution is acidified and the nonisotopic *p*-tolyloxyacetic acid is collected in 28% yield. After recrystallization from ether-petroleum ether the colorless prisms melt at 138–139° (dec.).

B. Notes

1. This mixing of reagents is inverse to the usual order.

2. The melting point is 34–35°, after distillation at 150–151° (10–11 mm.) and recrystallization from ether-petroleum ether.

3. The yield of product purified by distillation at 130–131° (10–11 mm.) is at least 85%.

4. The yield of product in trial runs was at least 88%, b.p. 112–114° (0.4 mm.).

5. The yield in trial runs was over 93%. After repeated recrystallization from alcohol the compound melts at 176–177°.

6. The mechanism of quaternary ammonium hydroxide decomposition has been studied extensively by Ingold.[1]

7. A similar degradative scheme is outlined by Ryan,[2] the first step hydroxylation being effected with performic acid by the method of Swern.[3]

8. Further degradation by periodic acid oxidation [see (6-methoxy-*m*-tolyl)acetaldehyde-2-C^{14}] yields nonisotopic *p*-tolyloxyacetaldehyde and formaldehyde-C^{14} (dimedon, 63.5% yield), having the same molar specific activity as the starting material. Ryan[2] has oxidized the aldehyde with excess silver oxide, then converted the formic-C^{14} acid to carbon-C^{14} dioxide with mercuric oxide.

9. The compound distills at 0.05 mm. with an air-bath temperature of 70–80°; yield 70–75%.

10. Traces of impurity lower the melting point very considerably.[4] Repeated recrystallization from absolute ether raises the melting point to 70°, b.p. 185–187° (15 mm.).

11. Schmid[5] has obtained the latter glycol in 80.5% yield [m.p. 49.5–50.5°, b.p. 100–105° (air-bath) at 0.03 mm.], by degradation of the ether with osmium tetroxide; see 3-(6-methoxy-*m*-tolyl)-1,2-propanediol-3-C^{14}. The yield by permanganate oxidation (6 oxygen atoms) at 0° was 27%.

C. Other Preparations

In a similar manner starting with phenol and 3-chloro-1-propanol-1-C^{14} are prepared:[2,6] 3-phenoxy-1-propanol-1-C^{14}, 3-bromopropyl-3-C^{14} phenyl

ether (*via* PBr_3),[2] 3-chloropropyl-3-C^{14} phenyl ether,[6] trimethyl-3-phenoxypropyl-1-C^{14}-ammonium bromide, trimethyl-3-phenoxypropyl-1-C^{14}-ammonium iodide (76.5% yield, m.p. 172–173°), and allyl-3-C^{14} phenyl ether (b.p. 88–90° at 22 mm., n_D^{25} 1.5200;[2] b.p. 72–74° at 10 mm., n_D^{20} 1.5209,[6] yield 87.5%).

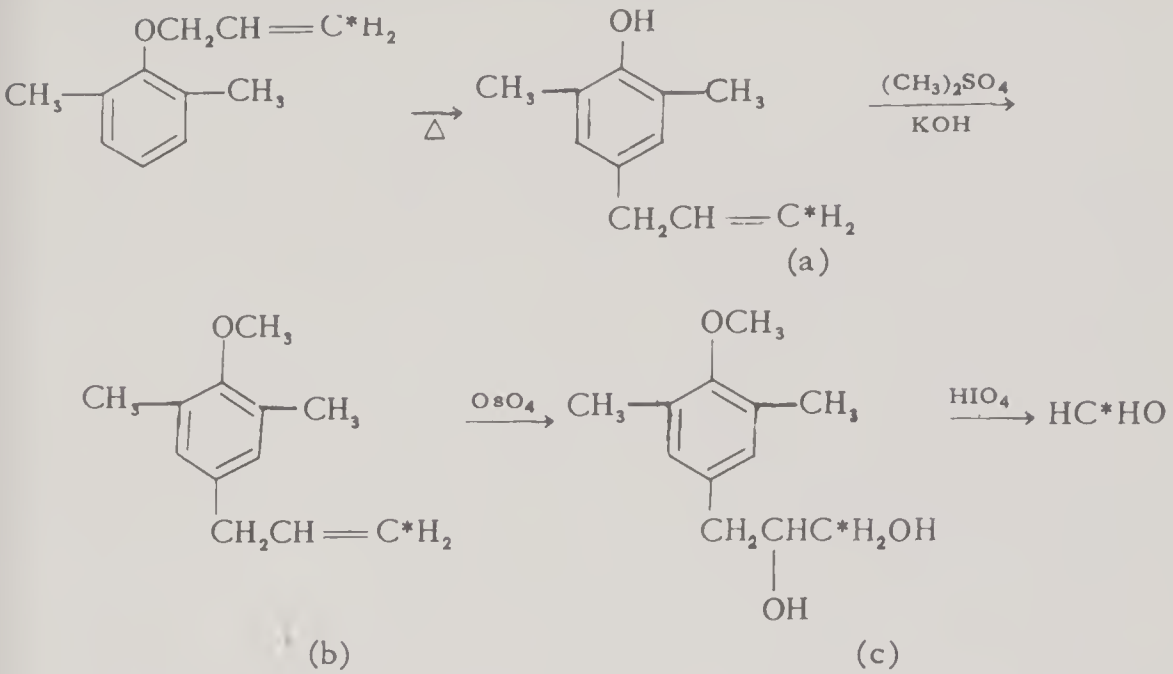
Likewise, from 2,6-xylenol are prepared:[2,5] 3-(2,6-xylyloxy)-1-propanol-1-C^{14} (Note 9, b.p. 144–146° at 13 mm., n_D^{24} 1.5170), 3-bromopropyl-3-C^{14} 2,6-xylyl ether (b.p. 140–142° at 12 mm., n_D^{24} 1.5290), 3-chloropropyl-3-C^{14} 2,6-xylyl ether (yield 89%, distills at 0.06 mm. with an air-bath at 95–105°), 3-iodopropyl-3-C^{14} 2,6-xylyl ether, trimethyl-3-(2,6-xylyloxy)-propyl-1-C^{14}-ammonium bromide or iodide (82% yield, m.p. 232–233.5°, from alcohol) and allyl-3-C^{14} 2,6-xylyl ether (b.p. 90–94° at 13 mm., n_D^{25} 1.5053, yield 90%).

Degradation of the allyl ethers shows that there is no isotopic rearrangement (Notes 7 and 8). The glycols formed are 3-phenoxy-1,2-propanediol-1-C^{14} (m.p. 62–64°, Note 10) and 3-(2,6-xylyloxy)-1,2-propanediol-1-C^{14} (m.p. 48–49°, Note 11).

The preparation of 3-chloro-1-propanol-1-C^{14}, by a similar method, has been reported by Ryan.[2] Sodium cyanide-C^{14} was condensed with ethylene oxide, and the resulting hydracrylonitrile-1-C^{14} was hydrolyzed in the manner described. 3-Chloropropionic-1-C^{14} acid was reduced directly to the carbinol with lithium aluminum hydride in 25–35% yield.

[1]W. Hanbart and C. K. Ingold, J. Chem. Soc., *1927*, 997.
[2]J. P. Ryan and P. R. O'Connor, J. Am. Chem. Soc., *74*, 5866 (1952).
[3]D. Swern, G. N. Billen and J. T. Scanlan, J. Am. Chem. Soc., *68*, 1506 (1946).
[4]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 297.
[5]H. Schmid and K. Schmid, Helv. Chim. Acta, *36*, 489 (1953).
[6]K. Schmid, W. Haegele and H. Schmid, Helv. Chim. Acta, *37*, 1080 (1954).

3,5-DIMETHYLESTRAGOLE-γ-C^{14}



H. Schmid and K. Schmid, Helv. Chim. Acta, *36*, 489 (1953).

A. Procedure (Note 1)

(a) 2,6-*Dimethylchavicol-*γ-C^{14}, *(4-Allyl-3-*C^{14}*-2,6-xylenol).* Allyl-3-C^{14} 2,6-xylyl ether (0.568 g.) is heated for 8 hours at 167.7° (phenetole-bath) in a small evacuated, sealed, Pyrex bomb tube. The mixture is taken up in petroleum ether and extracted with 20% potassium hydroxide. The extract is washed with petroleum ether, cooled, acidified and extracted with ether. The product is isolated by distillation at 10 mm. with an air-bath at 115–120°; yield 0.534 g. (94%), n_D^{25} 1.5368.

(b) *3,5-Dimethylestragole-*γ-C^{14}, *(4-Allyl-3-*C^{14}*-2,6-dimethylanisole).* The phenol (0.480 g.) is methylated at 90° with 1.18 g. of potassium hydroxide in 6 ml. of water and 1.33 g. of methyl sulfate, added in small portions according to the preparation of 4-methyl-o-estragole-α-C^{14}. The yield of colorless liquid is 0.429 g. (82.5%) b.p. 100–105° (air-bath temperature) at 10 mm. (Note 2).

(c) *3-(4-Methoxy-3,5-xylyl)-1,2-propanediol-1-*C^{14}. A solution of 0.429 g. of the methyl ether in 8 ml. of absolute ether is treated with 0.623 g. of osmium tetroxide in 20 ml. of absolute ether and 0.49 ml. of dry pyridine, then left overnight at 20°. The solvent is evaporated under reduced pressure, and the residue is treated with 0.196 g. of sodium hydroxide and 0.900 g. of mannitol in chloroform-water; see 3-(6-methoxy-*m*-tolyl)-1,2-propanediol-3-C^{14}. The crude product, obtained by distillation at 0.05 mm. with an air-bath at 125–130° (yield 0.376 g., 74%), is purified by recrystallization from ether-petroleum ether, m.p. 81–82.5°

(Note 3). Degradation studies indicate that there is no isotopic rearrangement (Note 4).

B. Notes

1. The synthesis is a study of the mechanism of the classical thermal *para* Claisen rearrangement[1] of the allyl ether of a phenol (or enol) to the isomeric C-allyl compound (without α-γ inversion of the allyl group). The isotopic study has been reported also by Ryan.[2] For the *ortho* rearrangement, see 2-allyl-1-C^{14}-*p*-cresol.

2. B.p. 113–114° (11 mm.), n_D^{30} 1.5182.[2] Permanganate oxidation[2] formed nonisotopic methoxytrimesic acid.

3. The isotopic compound, m.p. 81–82°, has been prepared by Ryan,[2] through hydroxylation of the ether with performic acid.[3] The preparation by permanganate oxidation (4–5 oxygen atoms) at 0° gives yields of 30–18%, in addition to an acid mixture (20–26%).

4. Directions are given by Schmid for cleaving the carbinol with periodic acid, forming formaldehyde-C^{14} (78% yield, as the dimedon derivative) and nonisotopic (4-methoxy-3,5-xylyl)acetaldehyde (89% yield). The molar specific activity of the formaldehyde-C^{14} dimedon is 97.9% of that of the unrearranged starting allyl ether. The rearrangement of an equimolecular mixture of allyl-3-C^{14} 2,6-xylyl ether and methyl 2-(allyloxy)-*m*-toluate gave nonisotopic methyl 5-allyl-2,3-cresotate, thus indicating the intramolecular nature of the rearrangement by the absence of cross-products.

C. Other Preparations

In a similar manner from allyl-3-C^{14} phenyl ether (Note 1) are prepared:[4] *o*-allyl-1-C^{14}-phenol, b.p. 104° (14–15 mm.), 93.5% yield, n_D^{20} 1.5428; *o*-estragole-α-C^{14}, b.p. 90–95° (air-bath temperature) (10 mm.), 93% yield; 3-(*o*-methoxyphenyl)-1,2-propanediol-3-C^{14}, b.p. 100–110° (air-bath temperature) (0.1 mm.), m.p. 60–61.5°, 78% yield; and formaldehyde. Directions are given[2,4] for degrading the allylanisole with alkaline permanganate, forming *o*-anisic-C^{14} acid, m.p. 100–101°, having the same molar specific activity. Ryan[2] has described similar preparations: *o*-estragole-α-C^{14}, b.p. 87–89.5° (13 mm.), n_D^{26} 1.5218; and 3-(*o*-methoxyphenyl)-1,2-propanediol-3-C^{14}, m.p. 62–63°.

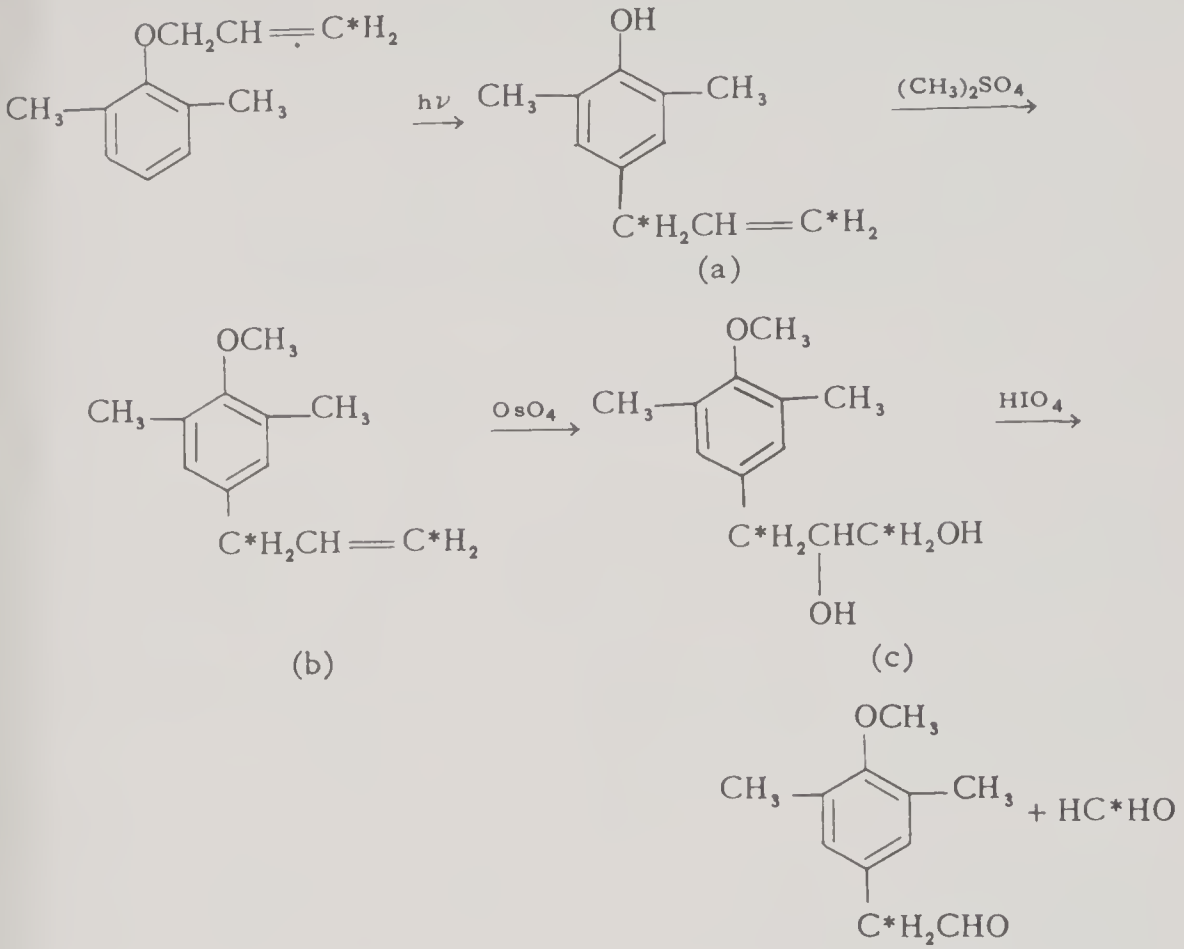
[1] D. S. Tarbell, Chem. Revs., 27, 495 (1940); *Organic Reactions*, Vol. II, Wiley, New York, 1944, Chapter 1; H. Conroy and R. A. Firestone, J. Am. Chem. Soc., 75, 2530 (1953); S. J. Rhodes, R. Raulins and P. D. Reynolds, *ibid.*, 75, 2531 (1953).

[2] J. P. Ryan and P. R. O'Connor, J. Am. Chem. Soc., 74, 5866 (1952).

[3] D. Swern, G. N. Billen and J. T. Scanlan, *ibid.*, 68, 1506 (1946).

[4] K. Schmid, W. Haegele and H. Schmid, Helv. Chim. Acta, 37, 1080 (1954).

2,6-DIMETHYLESTRAGOLE-α,γ-$C^{14}_{1/2}$



K. Schmid and H. Schmid, Helv. Chim. Acta, 36, 687 (1953); H. Schmid, Angew. Chem., 66, 108 (1954).

A. Procedure (Note 1)

(a) *2,6-Dimethylchavicol-α,γ-$C^{14}_{1/2}$, (4-Allyl-1,3-$C^{14}_{1/2}$-2,6-xylenol).* Allyl-3-C^{14} 2,6-xylyl ether (1.655 g., Note 2), under nitrogen in a quartz flask cooled in an air stream, is irradiated with ultraviolet light for a total of 58 hours at 25° (Note 3). At 18 hours the oil is dissolved in low-boiling petroleum ether containing 0.128 g. of carrier 2,6-dimethylchavicol and extracted with sodium hydroxide (20 g./100 ml. of water). The alkaline solution is washed with three portions of petroleum ether; then the combined petroleum ether solution and washes are washed with water and sodium chloride solution, dried, and concentrated by distillation through a column. The residue is distilled at 10 mm., and the unreacted starting material (1.168 g., 70.5%) is collected at 95–120° (air-bath temperature) and returned to the photolysis flask. The distillation residue is 0.193 g. (11.7%) of polymeric material. The process is repeated at 20-hour intervals, with the addition of 41 and 37.5 mg. of carrier, giving a final recovery of 48.8% of the starting material, 0.393 g. (23.4%) of polymeric

neutral material and about 25% phenolic product. The alkaline solution is cooled, acidified and extracted with petroleum ether; then the phenolic fraction is freed from insoluble resin by repetition of the process with 25 g. of sodium hydroxide in 75 ml. of water. The latter petroleum ether extract is concentrated and fractionally distilled at 10 mm., giving 0.1184 g. of 2,6-xylenol (Note 4), distilling at 90–110° (air-bath). The combined washes and original petroleum ether solution are extracted with a solution of 20 g. of sodium hydroxide in 100 ml. of water, and the extract is washed with petroleum ether, acidified and worked up as above; yield 0.123 g. (1.4%), b.p. 110–115° (air-bath temperature) (10 mm.). Degradation studies (see below) indicate that about 53% of the isotope is located at C-1.

(b) *2,6-Dimethylestragole-α,γ-$C^{14}_{1/2}$*, *(4-Allyl-1,3-$C^{14}_{1/2}$-2,6-dimethylanisole)*. A mixture of the C-allylphenol, 0.0523 g. of carrier and the residue obtained by evaporating the mother liquor (Note 4) is methylated at 90° with 0.740 g. of methyl sulfate and 0.656 g. of potassium hydroxide in 6 ml. of water; see 4-methyl-*o*-estragole-α-C^{14}. The product is dissolved in petroleum ether, and the solution is washed with 20% sodium hydroxide and hydrochloric acid, then distilled; yield 0.1564 g.

(c) *3-(4-Methoxy-3,5-xylyl)-1,2-propanediol-1,3-$C^{14}_{1/2}$*. To a solution of the methyl ether in 4 ml. of absolute ether is added 0.210 g. of osmium tetroxide and 0.17 ml. of anhydrous pydidine. After 4 hours, the solvent is decanted, and the adduct is washed with ether and decomposed with mannitol-sodium hydroxide-chloroform [see 3-(6-methoxy-*m*-tolyl)-1,2-propanediol-3-C^{14}].

The product is obtained by distillation in 0.1503 g. yield, b.p. 120–130° (air-bath temperature) at 0.05 mm. (Note 5).

B. Notes

1. The method is that of Kharasch[1] for the photochemical *para* rearrangement of phenyl ethers. The reaction is unique in that it is entered into by some ethers which have never undergone the *para*-Claisen rearrangement. The mechanism is believed to involve resonance of the free radical $\cdot CH_2CH{=}C^*H_2 \rightleftharpoons CH_2{=}C^*HCH_2\cdot$.

2. The phenyl allyl ether was purified previously in pentane solution by filtering through 50 g. of active basic aluminum oxide. The isotopic distribution is 97.4% at C-1.

3. A Hanau laboratory immersion lamp S81 is used unfiltered at 4 cm.

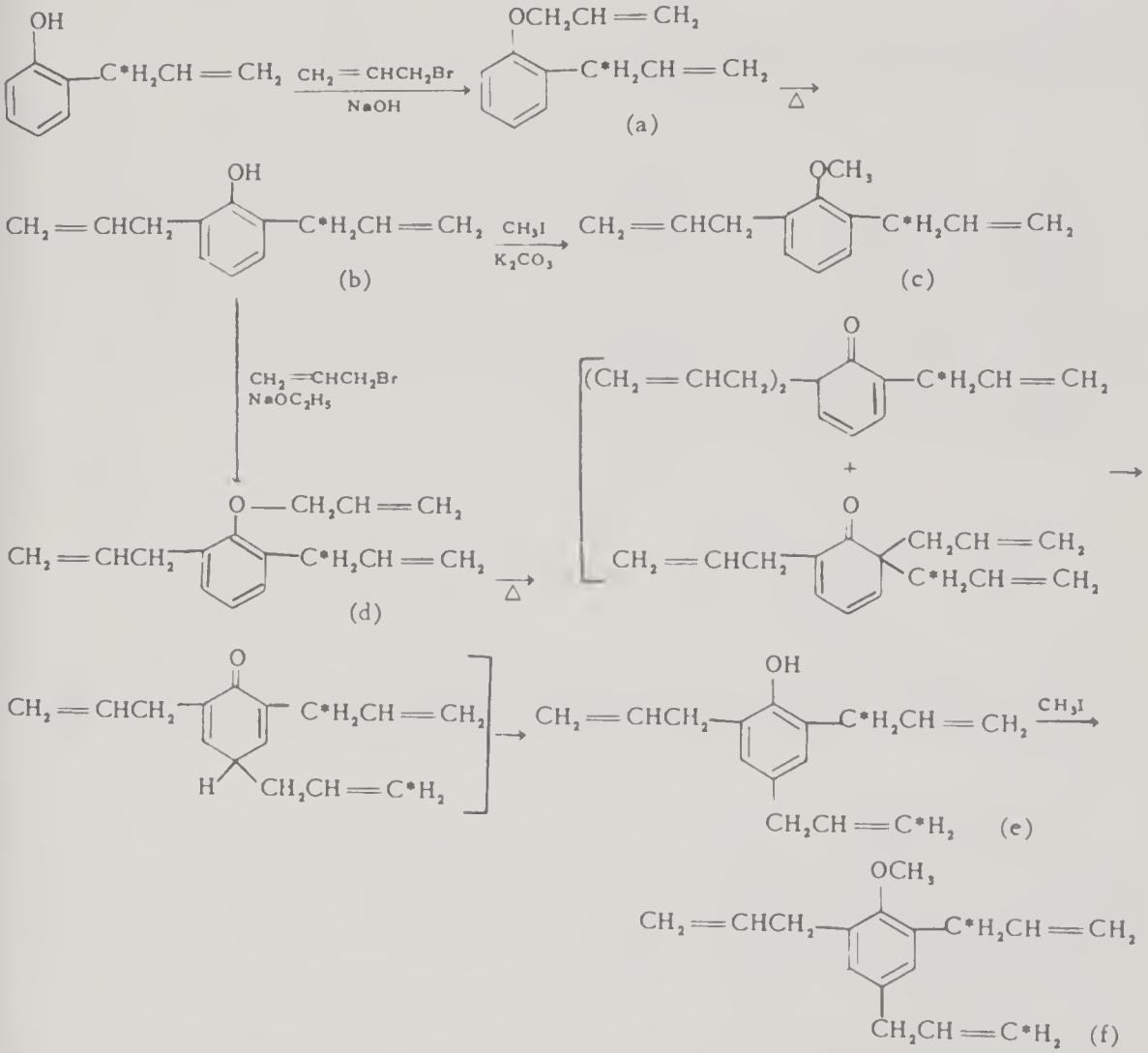
4. Recrystallization from ether-pentane gives 0.109 g. (8.8%) of 2,6-xylenol, m.p. 39–40°.

5. The melting point is 82–83°, after recrystallization to constant specific activity from ether-pentane. The glycol is degraded with periodic acid to formaldehyde-C^{14} (dimedon, m.p. 190–191°) and (4-methoxy-3,5-

xylyl)acetaldehyde-2-C^{14} (semicarbazone, m.p. 186–188°), having molar specific activities, respectively, of 51.8% and 45.9% of that of the glycol.

[1]M. S. Kharasch, G. Stampa and W. Nudenberg, Science, *116*, 309 (1952).

2-ALLYL-1-$C^{14}_{1/1}$-6-ALLYLESTRAGOLE-γ-$C^{14}_{1/1}$
[2-Allyl-1-$C^{14}_{1/1}$-4-allyl-3-$C^{14}_{1/1}$-6-allylanisole]



K. Schmid, W. Haegele and H. Schmid, Helv. Chim. Acta, *37*, 1080 (1954); Experientia, *9*, 414 (1953).

A. Procedure (Note 1)

(a) *Allyl 2-Allyl-1-C^{14}-phenyl Ether.* To a stirred mixture of 4.341 g. of 2-allyl-1-C^{14}-phenol, 1.30 g. of sodium hydroxide, 6 ml. of acetone and 6 ml. of water is added dropwise 5.10 g. of allyl bromide. The mixture is heated for 5 hours at 80–90°, stored overnight at 20° and extracted with petroleum ether. The extract is washed with 20% sodium hydroxide,

water, dilute hydrochloric acid, and sodium bicarbonate solution, then is dried, filtered through aluminum oxide and evaporated; yield 4.56 g., 81% (Note 2).

(b) *2-Allyl-1-C^{14}-6-allylphenol.* A mixture of 7.545 g. of allyl 2-allyl-1-C^{14}-phenyl ether, and 8 ml. of *N,N*-diethylaniline in an evacuated sealed tube is heated at 200° for 7 hours. The mixture is dissolved in petroleum ether, and the solution is washed with dilute sulfuric acid and extracted with Claisen's alkali (Note 3). The alkali is washed with petroleum ether, then cooled and acidified with hydrochloric acid. The product is isolated by extraction with ether and distilled (Note 4); yield 6.848 g. (91%), n_D^{20} 1.5389, b.p. 129–130° (12.5 mm.).

(c) *6-Allyl-o-estragole-α-C^{14}, (2-Allyl-1-C^{14}-6-allylanisole).* A stirred mixture of 0.775 g. of 2-allyl-1-C^{14}-6-allylphenol 6.4 g. of calcined potassium carbonate, 3.2 g. of methyl iodide and 20 ml. of dry acetone is refluxed for 48 hours in the dark. The mixture is diluted with water, and extracted with light petroleum, and the extract is washed with Claisen's alkali (Note 3), water, dilute hydrochloric acid and salt solution. The product is collected by distillation (Note 4) with an air-bath at 110–120° (10 mm.); yield 0.801 g., 96% (Note 5).

(d) *Allyl 2-Allyl-1-C^{14}-6-allylphenyl Ether.* To a stirred mixture at 90° containing 5.434 g. of 6-allyl-o-estragole-α-C^{14}, and a solution of 0.720 g. of sodium in 20 ml. of absolute alcohol is added dropwise 4.75 g. of freshly distilled allyl bromide. The mixture is heated for 5 hours, then stored overnight at 25° and extracted with light petroleum. The extract is washed with Claisen's alkali (Note 3) and water, dried, filtered through aluminum oxide and evaporated; yield 5.402 g. (81%). Purification is accomplished by vacuum distillation, n_D^{20} 1.5229 (Notes 4 and 6).

(e) *2-Allyl-1-C$^{14}_{1/1}$-6-allylchavicol-γ-C$^{14}_{1/1}$,* [*2-(Allyl-1-C$^{14}_{1/1}$)-4-(allyl-3-C$^{14}_{1/1}$-6-allylphenol*] (Note 7). Allyl 2-allyl-1-C^{14}-6-allylphenyl ether (2.90 g.) is rearranged by heating in diethylaniline for 5 hours as described above; yield 2.302 g. (80%), distilled at an air-bath temperature of 85–95° at 0.1 mm. (Note 7).

(f) *2-Allyl-1-C$^{14}_{1/1}$-6-allylestragole-γ-C$^{14}_{1/1}$* (Note 7). The phenol (2.302 g.) is methylated with 8.5 g. of methyl iodide in the manner described above. The yield of product, distilled at 0.15 mm. with an air-bath at 75–85°, is 2.417 g. (97.5%), n_D^{20} 1.5225. Degradation studies indicate that 70% of the isotope is located at the allyl-1-C^{14}-position (Note 8).

B. Notes

1. These syntheses are incidental to a study of the mechanism of the *para*-Claisen rearrangement. Also see 3-(3,5-dimethyl-4-methoxyphenyl)-propanediol-1-C^{14}, Note 1.

2. B.p. 108–109° (11 mm.), n_D^{20} 1.5231.

3. This solvent for weakly acidic (highly substituted) phenols is prepared by diluting a solution of 350 g. of potassium hydroxide and 250 ml. of water to 1000 ml. with methanol.

4. The distillation residue is in turn distilled in a tube with bulbar enlargements.

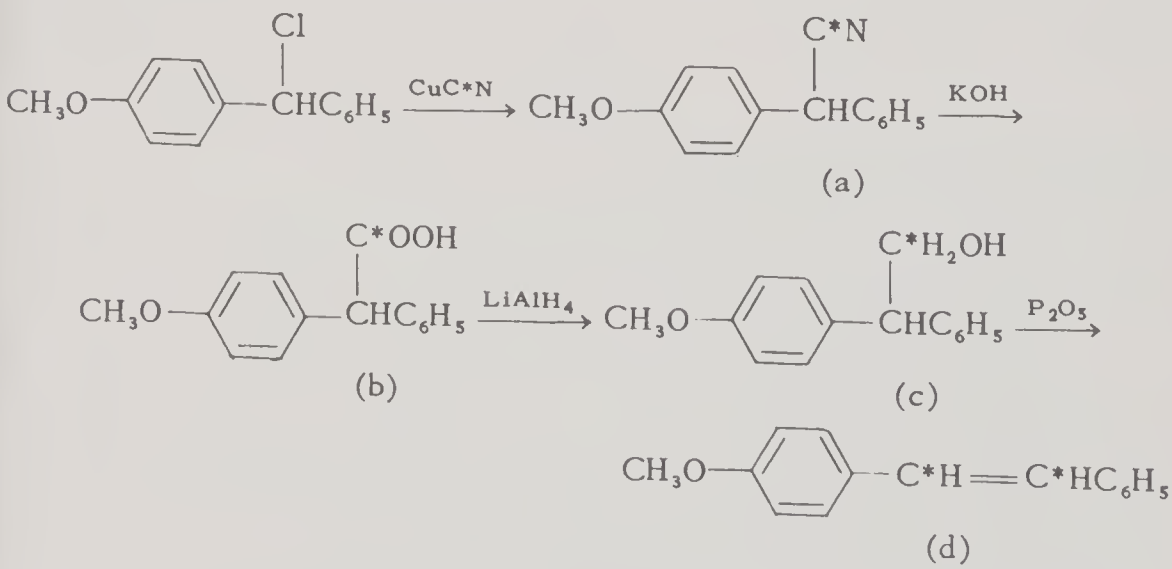
5. Directions are given by Schmid for degrading the compound with alkaline permanganate, forming 2-methoxyisophthalic-C_1^{14} acid, m.p. 219–221°, with a molar specific activity of 91% of that of the starting material.

6. A trial preparation distilled at 78–81° (0.2 mm.). A sample hydrogenated at room temperature and pressure, in ethyl acetate over palladium, absorbed 2.94 moles of hydrogen.

7. A trial preparation distilled at 94° (0.1 mm.), n_D^{20} 1.5408.

8. Directions are given by Schmid for oxidizing this ether with alkaline permanganate, then esterifying the C_1^{14}-trimesic acid, (3,5-dicarboxy-*o*-anisic-C^{14} acid), with diazomethane. The methyl 3,5-dicarbomethoxy-*o*-anisate-C^{14}, m.p. 84–85°, possesses 70% of the molar specific activity of the starting material. Ozonolysis of the ether forms formaldehyde-C^{14}, which has a molar specific activity of 9.89% of that of the starting material.

4-METHOXYSTILBENE-α,α′-$C_{1/2}^{14}$



J. G. Burr, Jr. and L. S. Ciereszko, J. Am. Chem. Soc., 74, 5426 (1952).

A. Procedure

(a) (p-*Methoxyphenyl*)*phenylacetonitrile-1-*C^{14}. A mixture of 8.3 g. of *p*-α-chlorobenzylanisole (Note 1) and 3.2 g. of cuprous cyanide-C^{14} (dried at 110°) is heated with a preheated oil bath at 180°, and during the

course of 1 hour the temperature is raised to 190°. The reaction mixture is extracted with chloroform, and the filtered extract is concentrated, diluted with hexane and cooled. The first crop of product is 2.8 g., m.p. 130°, and the filtrate yields an additional crop, making the yield 4.8 g., 71% (Note 2).

(b) *(p-Methoxyphenyl)phenylacetic-1-C^{14} Acid.* A mixture of 2.85 g. of the nitrile, 15 g. of potassium hydroxide, 15 ml. of water and 50 ml. of ethanol is refluxed for 2 days. The acidic product is 3.08 g. of light brown oil. Repeated extraction with boiling hexane yields 2.37 g. (68%) of colorless crystalline product, m.p. 96° (Note 3).

(c) *2-(p-Methoxyphenyl)-2-phenylethanol-1-C^{14}.* The (*p*-methoxyphenyl)-phenylacetic-1-C^{14} acid (2.00 g.) is refluxed for 1 hour with a solution of 2.0 g. of lithium aluminum hydride in 75 ml. of ether. The excess reagent is destroyed by cautious addition of ethyl acetate; then sodium hydroxide (10%) is added until a dry, granular precipitate of aluminum hydroxide is just formed. Filtration and evaporation give 1.75 g. (93%) of colorless oil.

(d) *4-Methoxystilbene-α,α'-$C^{14}_{1/2}$.* The 2-(*p*-methoxyphenyl)-2-phenylethanol-1-C^{14} is heated for 15 minutes with a mixture of phosphorus pentoxide in 25 ml. of dry xylene. The solvent is decanted, the flask is rinsed with ether, and the combined solution is decolorized with carbon and evaporated. The residue is crystallized from hexane to give 0.65 g. of colorless prisms, m.p. 135°; more is obtainable from the mother liquor. The molar specific activity is the same as that of the starting material. Degradation studies indicate that 95.5% of the activity is at C-α, the carbon adjacent to the *p*-methoxyphenyl group (Note 4).

A series of other substituted stilbene-α,α'-$C^{14}_{1/2}$-compounds is prepared by these same procedures:

1. Phenyl-*p*-tolyl-acetonitrile-1-C^{14}, 64%, m.p. 60–61°.
   Phenyl-*p*-tolyl-acetic-1-C^{14} acid, 89%, m.p. 110–112°.
   2-Phenyl-2-(*p*-tolyl)ethanol-1-C^{14}, 93%, m.p. 43–44°.
   4-Methylstilbene-α,α'-$C^{14}_{1/2}$, 100%, m.p. 120° (Note 5).
2. (*p*-Ethylphenyl)phenylacetonitrile-1-C^{14}, 90%, oil.
   (*p*-Ethylphenyl)phenylacetic-1-C^{14} acid, 65%, m.p. 78°.
   2-(*p*-Ethylphenyl)-2-phenylethanol-1-C^{14}, 95%, oil (Note 6).
   4-Ethylstilbene-α,α'-$C^{14}_{1/2}$, 100%, m.p. 82° (Note 7).
3. *p*-Cumenyl-phenylacetonitrile-1-C^{14}, 86%, oil.
   p-Cumenyl-phenylacetic-1-C^{14} acid, 54%, oil (Note 8).
   2-(*p*-Cumenyl)-2-phenylethanol-1-C^{14}, 92%, oil.
   4-Isopropylstilbene-α,α'-$C^{14}_{1/2}$, 100%, m.p. 80° (Note 9).
4. (*p-t*-Butylphenyl)phenylacetonitrile-1-C^{14}, 76%, m.p. 98°.
   (*p-t*-Butylphenyl)phenylacetic-1-C^{14} acid, 57%, m.p. 139°.
   2-(*p-t*-Butylphenyl)-2-phenylethanol-1-C^{14}, 100%, m.p. 82°.
   4-*t*-Butylstilbene-α,α'-$C^{14}_{1/2}$, 100%, m.p. 96° (Note 10).

5. Phenyl-3,4-xylylacetonitrile-1-C^{14}, 70%, m.p. 54°.
   Phenyl-3,4-xylylacetic-1-C^{14} acid, 70%, m.p. 124°.
   2-Phenyl-2-(3,4-xylyl)ethanol-1-C^{14}, 90%, oil (Note 11).
   3,4-Dimethylstilbene-α,α'-$C^{14}_{1/2}$, 100%, m.p. 78° (Note 12).
6. Phenyl-*m*-tolylacetonitrile-1-C^{14}, 80%, m.p. 26°, n_D^{25} 1.5760.
   Phenyl-*m*-tolylacetic-1-C^{14} acid, 93%, m.p. 122°.
   2-Phenyl-2-(*m*-tolyl)ethanol-1-C^{14}, 92%, oil (Note 13).
   3-Methylstilbene-α,α'-$C^{14}_{1/2}$, 100%, m.p. 49–50° (Note 14).
7. (4-Biphenylyl)phenylacetonitrile-1-C^{14}, 70%, m.p. 131–132°.
   (4-Biphenylyl)phenylacetic-1-C^{14} acid, 88%, m.p. 143–144°.
   2-(4-Biphenylyl)-2-phenylethanol-1-C^{14}, 88%, m.p. 100–101°.
   4-Phenylstilbene-α,α'-$C^{14}_{1/2}$, 100%, m.p. 222° (Note 15).
8. Mesitylphenylacetonitrile-1-C^{14},[1] 71%, m.p. 90°.
   Mesitylphenylacetic-1-C^{14} acid,[1] 56%, m.p. 176°.
   2-Mesityl-2-phenylethanol-1-C^{14},[1] 49%, m.p. 76° (Note 16).
9. Phenyl-*o*-tolylacetonitrile-1-C^{14}, 83%, b.p. 109–111° (0.06–0.08 mm.).[2]
   Phenyl-o-tolylacetic-1-C^{14} acid (Note 17).[2]
   2-Phenyl-2-(o-tolyl)ethanol-1-C^{14}, 80%, m.p. 58.5–59°.[2]
   2-Methylstilbene-α,α'-$C^{14}_{1/2}$, 100%, oil (Note 18).
10. Stilbene-α-C^{14}, m.p. 125–126° (Note 19).
11. Phenylacetonitrile-1-C^{14}, 72%, b.p. 100–101° (8.5 mm.).[3]
12. *p*-Methoxyphenyl acetonitrile-1-C^{14}, 67%, b.p. 95.5–97° (0.7 mm.).[3]

B. Notes

1. Substituted benzhydryl chlorides are prepared conveniently by reduction of the benzophenones with zinc dust-sodium hydroxide, followed by treatment of the benzhydrols with thionyl chloride; see α-phenyl-1-naphthaleneacetonitrile-C^{14}. Budde and Potempa[4] have successfully reacted the benzhydryl bromides with cuprous cyanide.

2. Recrystallization does not raise the melting point above 130°.

3. Hydrolysis of these nitriles is effected usually by boiling in 70% sulfuric acid; see α-phenyl-2-naphthaleneacetic-C^{14} acid. Acid hydrolysis for 1–6 hours, however, produces only neutral gums in this instance.

4. Directions are given by Burr and Ciereszko (also see Bonner)[5] for the permanganate oxidation of the stilbene to a mixture of benzoic-C^{14} acid and *p*-anisic-C^{14} acid. Demethylation with aluminum bromide and extraction with carbon disulfide leave a residue of *p*-hydroxybenzoic-C^{14} acid, m.p. 213–214° from dilute alcohol; the molar specific activity is 95.5% of that of the starting materials.

5. The carbon adjacent to the *p*-tolyl group contributes 66.4% of the specific activity. Experiment shows that the degree of *p*-tolyl migration

is relatively insensitive to wide variation in the conditions of rearrangement of the carbinol. The reaction has been shown to be irreversible.

6. 1-Naphthylcarbamate; m.p. 92°.

7. The carbon adjacent to the *p*-ethylphenyl group contributes 68.6% of the specific activity.

8. Amide, m.p. 97°; anilide, m.p. 211°.

9. α,α′-Dibromo-4-isopropylbibenzyl-α,α′-$C^{14}_{1/2}$, m.p. 228°. The carbon adjacent to the *p*-cumenyl group contributes 64.5% of the specific activity.

10. α,α′-Dibromo-4-*t*-butylbiphenyl-α,α′-$C^{14}_{1/2}$, m.p. 230°. The carbon adjacent to the *p*-*t*-butylphenyl group contributes 76.3% of the specific activity. Refluxing the stilbene with chromium trioxide in acetic acid solution for 20 minutes gives pure *p*-*t*-butylbenzoic-C^{14} acid, m.p. 165°.

11. 1-Naphthylcarbamate, m.p. 149°.

12. The carbon adjacent to the 3,4-xylyl group contributes 65.6% of the specific activity.

13. 1-Naphthylcarbamate, m.p. 118°.

14. α,α′-Dibromo-3-methylbiphenyl-α,α′-$C^{14}_{1/2}$, m.p. 178°. The carbon adjacent to the *m*-tolyl group contributes 61.3% of the specific activity.

15. The carbon adjacent to the 4-biphenylyl group contributes 57.2% of the specific activity.

16. 1-Naphthylcarbamate, m.p. 87° (indefinite). This carbinol could not be induced to rearrange[1] to the trimethylstilbene, nor did the comparable rearrangement of 2-mesityl-2-phenylethylamine-1-C^{14} take place with nitrous acid. The dehydration product probably was olefinic, but oxidation by an established procedure gave only a neutral oil.

17. The nitrile is hydrolyzed by refluxing for 2 hours with 70% sulfuric acid; yield nearly quantitative.[2] A sample recrystallized six times from cyclohexane melted at 96–97.6°.

18. α,α′-Dibromo-2-methylbibenzyl-α,α′-$C^{14}_{1/2}$, m.p. 160°. The carbon adjacent to the *o*-tolyl group contributes 45% of the specific activity. Directions are given by Benjamin[2] for the two-stage oxidation of 2-methylstilbene-α,α′-$C^{14}_{1/2}$ with peracetic acid, then with permanganate, forming benzoic-C^{14} acid and phthalic-C^{14}_1 acid. The latter is isolated as *N*-phenylphthalimide-C^{14}_1, m.p. 206–207°; the molar specific activity is 45% of that of the starting material.

19. The preparation[6] is by the Wagner rearrangement dehydration of 2,2-diphenylethanol-1-C^{14}. Stilbene-α-C^{14} has been reduced[5] to bibenzyl-α-C^{14}, m.p. 53.5–54°, by hydrogenation at ordinary temperature and pressure, using Raney nickel in benzene or Adams catalyst in alcohol. The isotopic compound is reduced 0.98 times as rapidly as the nonisotopic stilbene. There is no exchange of hydrogen between bibenzyl and stilbene-α-C^{14} in the presence of nickel catalyst, nor between stilbene and bibenzyl-α-C^{14} under hydrogenation conditions.

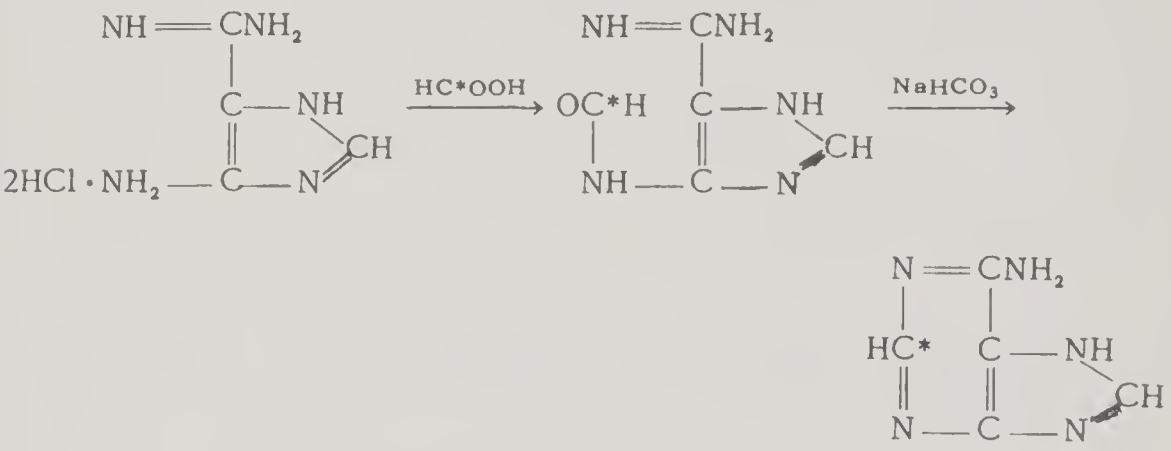
C. Other Preparations

4-Methyl-, 3-methyl-, 4-phenyl-, and 4-methoxystilbene-α,α'-$C^{14}_{1/2}$ have been formed[7] by a Demjanow rearrangement from the appropriate 2-aryl-2-phenylethylamine-1-C^{14}, followed by dehydration of the resulting secondary carbinol mixtures. Oxidative degradation studies indicate that the carbon atom adjacent to the substituted phenyl group (C-α) contributes, respectively, 47, 48, 50 and 59% of the specific activity of the stilbene. 2-Methylstilbene-α,α'-$C^{14}_{1/2}$ has been formed,[1] similarly, with 42% *o*-tolyl group migration (42% of the radioactivity at C-α).

[1]P. S. Bailey and J. G. Burr, Jr., J. Am. Chem. Soc., *75*, 2954 (1953).
[2]B. M. Benjamin and C. J. Collins, J. Am. Chem. Soc., *75*, 402 (1953).
[3]J. D. Roberts and C. M. Regan, J. Am. Chem. Soc., *75*, 2069 (1953); *Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 107.
[4]W. M. Budde and S. J. Potempa, J. Am. Chem. Soc., *74*, 258 (1952).
[5]W. A. Bonner and C. J. Collins, J. Am. Chem. Soc., *75*, 4516 (1953).
[6]W. A. Bonner and C. J. Collins, J. Am. Chem. Soc., *75*, 3693 (1953).
[7]L. S. Ciereszko and J. G. Burr, Jr., J. Am. Chem. Soc., *74*, 5431 (1952).

HETEROCYCLIC COMPOUNDS

ADENINE-2-C^{14}
(6-Aminopurine-2-C^{14})



A. R. P. Paterson and S. H. Zbarsky, J. Am. Chem. Soc., 75, 5753 (1953).

A. Procedure (Note 1)

A solution of 0.200 g. of 4(or 5)-amino-5(or 4)-imidazolecarboxamidine dihydrochloride[1] and 0.170 g. of potassium formate-C^{14} in 2 ml. of 20% formic acid (Note 2) is refluxed for 4 hours. Without isolation of the 4(or 5)-formamido-C^{14}-5(or 4)-imidazolecarboxamidine, the solution is diluted to 8 ml. with water; then sufficient potassium bicarbonate is added slowly to make the solution 0.5 *M* in bicarbonate. The solution is refluxed for 1 hour; then most of the alkali is neutralized with hydrochloric acid, and the solution is concentrated to 2–3 ml. under reduced pressure and stored in a refrigerator. The crude product is centrifuged, washed with three portions of ice water (Note 3), dried under vacuum and sublimed at 220° (1 mm); yield 0.083 g. (61%). The molar specific activity is about the same as that of the recovered formate-C^{14} (Note 4). Purity is checked by paper chromatography.[1,2]

B. Notes

1. The procedure is a modification of the method of Shaw,[1] altered to conserve formic acid.

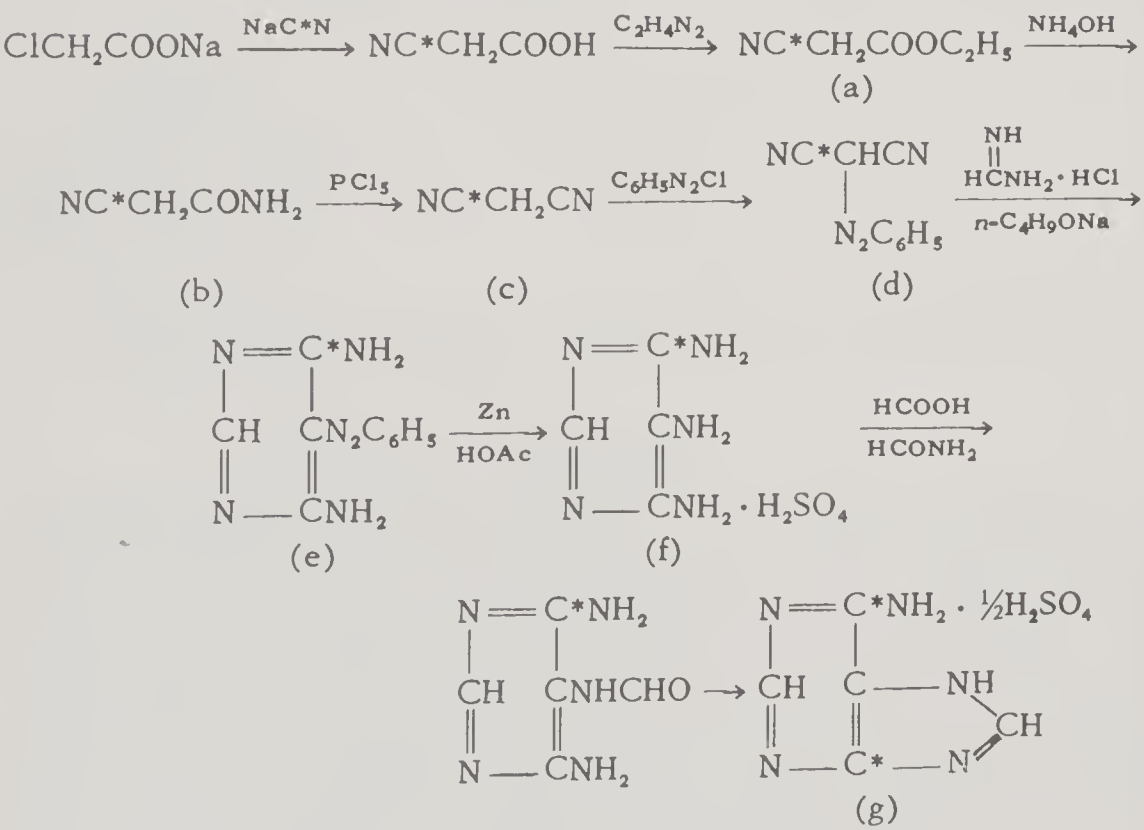
2. The reaction vessel may be made conveniently from the outer member of a 24/40 ⅋ joint. This mixture is 6.3 *M* formate; the yields drop to 40–42% in 4 *M* formate.

3. The combined liquors are saved for the nearly quantitative recovery of formate-C^{14} by steam distillation, titration with potassium hydroxide and evaporation to dryness.

4. The yield runs between 60% and 65% based on the carboxamidine; Shaw reports 80% yield. The product loses three molecules of water of crystallization at 54°, and the pearly crystals turn white. The picrate melts at 286–287° (dec.).

[1]E. Shaw, J. Biol. Chem., *185*, 439 (1950).
[2]J. D. Smith and R. Markham, Biochem. J., *46*, 509 (1950).

ADENINE-4,6-$C^{14}_{1/2}$ SULFATE



E. L. Bennett, J. Am. Chem. Soc., *74*, 2420 (1952); Nucleonics, 7 (4), 49, 50 (1950).

A. Procedure (Note 1)

(a) *Ethyl Cyano-C^{14}-acetate.* To the hydrogen cyanide-C^{14} prepared from 6 mmoles of barium carbonate-C^{14} (85% yield), and absorbed in 7.2

mmoles of 0.3 *N* sodium hydroxide, is added 34 mmoles of sodium cyanide solution (2 *M*) and 44 mmoles of sodium chloroacetate solution (2 *M*). The solution is heated at 70–95° over a 20-minute period, then cooled, acidified with perchloric (or sulfuric) acid and continuously extracted with ether for 48 hours. The ether solution of cyano-C^{14}-acetic acid is esterified by addition of an excess of diazoethane in ether at 0° (Note 2). The water is removed by freezing the solution at −70°, and the ether solution is decanted into two equal portions (Note 3). The solutions are concentrated by distillation through a Vigreux column at atmospheric pressure, then at reduced pressure until the residual volumes are about 5 ml. Benzene (50 ml.) is added, the aqueous phase is removed, and the product is dried by azeotropic distillation at 40 mm. (Note 4) to a residual volume of 5 ml. (60° bath temperature). The process is repeated with an additional 30 ml. of benzene.

(b) *2-Cyano-C^{14}-acetamide.* A mixture of the ethyl cyano-C^{14}-acetate and 3 ml. of concentrated ammonium hydroxide is stored for 30 minutes at room temperature and then at 0°. The product is filtered off, washed with cold alcohol and dried (0.800 g.). The filtrate is concentrated to 1 ml., diluted with 4 ml. of alcohol, chilled at 0° and processed as before. The combined yield is 1.27 g. (Note 5), 76% based on sodium cyanide.

(c) *Malononitrile-1-C^{14}* (Note 6). The 2-cyano-C^{14}-acetamide (1.27 g.) is mixed with 1.3 g. of phosphorus pentachloride in a microsublimation apparatus (Note 7). The dehydration is effected by heating the evacuated mixture (water aspirator) for 4 minutes at 95–100°. The cold finger is cooled to −70°, and the product is sublimed as quickly as possible (5 min.) at 30 mm. with a preheated oil-bath at 160–170°.

(d) *Phenylazomalononitrile-1-C^{14}.* To a solution of the malononitrile-1-C^{14} in 5 ml. of methanol and 22 ml. of 2 *M* sodium acetate is added a solution of benzenediazonium chloride, prepared by adding 1.75 g. of sodium nitrite to a mixture of 1.9 g. of aniline, 6.75 ml. of hydrochloric acid and 35 g. of ice. After storage at 0°, the yellow product is collected and washed with cold water (Note 8); yield 1.70 g., 66% based on cyanoacetamide.

(e) *4,6-Diamino-5-phenylazopyrimidine-4-C^{14}* (Note 9). A 1.0 *M* sodium butoxide solution (17 ml. in absolute butanol, Note 10) and 1.24 g. of formamidine hydrochloride (Note 11) are added to 10 mmoles of phenylazomalononitrile-1-C^{14}. The mixture is stirred for 10 minutes at room temperature and refluxed for 4 hours under anhydrous conditions. After storage at 0°, the solid is collected, washed with cold water and alcohol, and dried; corrected yield (Note 12), 1.4 g. (70%).

(f) *4,5,6-Triaminopyrimidine-4-C^{14} Sulfate.* To a solution of 1.6 g. of crude (85%) 4,6-diamino-5-phenylazopyrimidine-4-C^{14} in 10 ml. of glacial acetic acid is added 2.5 g. of powdered zinc in small portions during 15 minutes. The metal is filtered off and washed with three 4-ml. portions

of warm water containing 1 ml. of acetic acid. To the cooled combined solution is added 5 ml. of 9 *M* sulfuric acid and, after standing at 5°, the product is collected and washed with three 2-ml. portions of cold water; yield 1.23 g., 78% (Note 13).

(g) *Adenine-4,6-$C^{14}_{1/2}$ Sulfate* (Note 14). 4,5,6-Triaminopyrimidine-4-C^{14} sulfate (0.612 g.) is heated under reflux at 160° for 2.5 hours with a solution of 0.3 ml. of 98–100% formic acid in 11 ml. of formamide. An additional 0.25 ml. of formic acid is added after 1.2 hours. The solvent is distilled off under vacuum at 140–145°. The residue, *N*-(4,6-diamino-5-pyrimidinyl-4-C^{14})formamide, is dissolved in 9 ml. of warm *N* sulfuric acid, decolorized with charcoal and crystallized at 5°. The pale yellow product is collected, washed with a small amount of cold water and alcohol, then air-dried; yield 0.341 g., 67% (Note 15). The over-all yield is 20% based on sodium cyanide.

B. Notes

1. The procedure is a modification of the isotopic procedure described by Cavalieri.[1]

2. The reagent may be prepared conveniently, as needed, from 1-nitroso-1-ethylurea in the manner described by Arndt[2] for diazomethane.

3. Each portion contains about 18 mmoles of crude product.

4. Ethyl cyanoacetate boils at 97° (16 mm.); 107° (27 mm.). The yield[3] by a similar procedure on a large scale is of the order of 80%.

5. The product prepared in this manner[4] usually contains small amounts of malonamide (m.p. 170–171°), which may be removed by recrystallization from alcohol, producing a product melting at 119–120°.

6. The procedure is an adaptation of the method of Corson, Scott and Vose,[5] and is essentially that described by Cavalieri.[1] The phosphorus pentachloride method (67–80% yield) is superior to the phosphorus pentoxide method of dehydration, which leads to the formation of a black, sticky mass. The use of larger proportions of PCl_5 to amide than 5 to 2 allows the formation of considerable phosphorus oxychloride, with marked reduction in yield.[6] Gal and Shulgin[7] have raised the yield by the phosphorus pentoxide method to 85%, by adding 10% of powdered lithium chloride to the reaction mixture.

7. Bennett has presented a diagram of the sublimator which is equipped with a stopcock and a cold finger for Dry Ice cooling. The mixing is done in a dry-box.

8. The product may be recrystallized from benzene (5 ml. per g.), but is satisfactory for the next step. Cavalieri[1] has obtained phenylazomalononitrile in 20% yield based on 2-cyanoacetamide (55–60% based on malononitrile) by this adaptation of the method of Lythgoe.[8]

9. The procedure is essentially that described by Cavalieri.[1]

10. The yield of condensation product is 30–55% in ethanol and 0% in methanol,[1,9] although Baddiley[10] has described the preparation in ethanol in 75% yield, m.p. 282–286° (dec.), color reddish-brown.

11. Formamidine hydrochloride may be prepared in 90–100% yield by reaction of ethyl formimidate hydrochloride[1,9,10,11,12] with ammonia in a bomb tube, by the procedure of Cavalieri.[1] Bennett[9] has reported that the yield of ethyl formimidate hydrochloride is probably improved (65% on a pure basis) by reducing the molar excess of hydrogen chloride[12] to 10%.

12. The purity of the product is indicated to be 85% by chromatography on silicic acid-Celite, developed by 95% benzene-5% ethanol. As the compound is difficult to recrystallize, it is used without further purification. Cavalieri has reported a yield of 70–80% at the 0.12-mole scale.

13. The procedure is a modification of that described by Cavalieri (55–78% yield).[1] The salt ($C_4H_7N_5 \cdot H_2SO_4 \cdot H_2O$) may be recrystallized from 2 *N* sulfuric acid (20 ml./g.) with an 85% recovery.

14. The procedure is a modification of that of Cavalieri,[1] designed to eliminate explosions. The yield is lowered by refluxing the amine with formic acid, or by extending the heating period with formamide.

15. Additional product may be recovered by passing the mother liquor through an ion exchange column (Amberlite IRA-400 in the chloride form), concentrating the eluate to 2.5 ml. and adding 0.060 g. of carrier adenine sulfate. Sulfuric acid (0.1 ml., 18 *N*) is added, and the product is isolated as before (0.080 g.). The compound may be recrystallized from 2 *N* sulfuric acid as $(C_5H_5N_5)_2 \cdot H_2SO_4 \cdot H_2O$. Filter paper chromatography with 40% 1-butanol-25% propionic acid-35% water indicates the presence of only one radioactive compound (R_f 0.42 when applied to Whatman No. 1 paper from hydrochloric acid solution).

C. Other Preparations

Cavalieri[1] has described the preparation of adenine-4,6-$C^{14}_{1/2}$ sulfate, in in 3.8% over-all yield based on sodium cyanide, by a modification of the procedure described. 2-Cyano-C^{14}-acetamide was prepared (42% yield) by ammonolysis of methyl cyano-C^{14}-acetate, then converted to 4,6-diamino-5-phenylazopyrimidine-4-C^{14} essentially by the procedure described. 4,5,6-Triaminopyrimidine-4-C^{14} sulfate was prepared (55–78% yield) by a slight modification of the described procedure, then converted to *N*-(4,6-diamino-5-pyrimidinyl-4-C^{14})formamide (95% yield) by dissolution in 98% formic acid and evaporation at room temperature. Adenine-4,6-$C^{14}_{1/2}$ sulfate was prepared by heating the formamidopyrimidine with formamide at 170° for 2.5 hours in a sealed tube, and recrystallizing the

product from 2 *N* sulfuric acid; yield 77–87% based on 4,5,6-triaminopyrimidine sulfate. Bennett[8] has reported explosive difficulties with the sealed system.

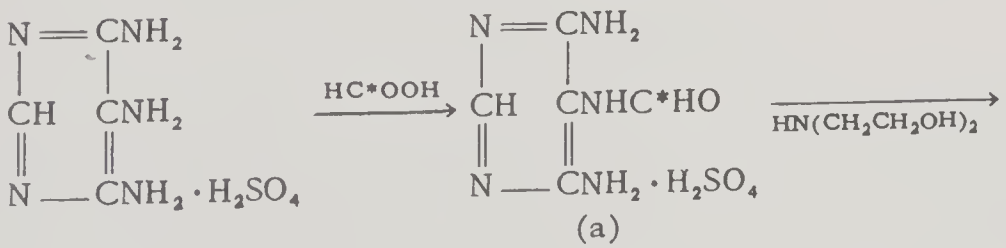
2-Cyanoacetamide-C_2^{14} has been prepared in 65% yield by Gal,[7] by esterifying cyanoacetic-C_2^{14} acid with methyl sulfate, and then submitting the methyl cyanoacetate-C_2^{14} to ammonolysis.

The preparation of malononitrile-1,2-C_2^{14} has been described by Gal.[7] A mixture of 1.00 g. of 2-cyanoacetamide-C_2^{14}, 2.5 g. of phosphorus pentoxide and 0.1 g. of lithium chloride was heated over an open flame, and, without delay, the product was distilled at 1 mm. (within 5 minutes) into a Dry Ice-cooled receiver; yield 0.672 g. (85.7%), m.p. 32°. The molar specific activity was 98.8% of that of the starting material.

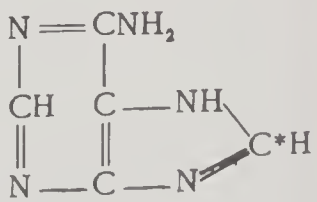
[1]L. F. Cavalieri, J. F. Tinker and A. Bendich, J. Am. Chem. Soc., *71*, 533 (1949).
[2]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 165.
[3]*Ibid.*, Coll. Vol. I, Wiley, New York, 1941, p. 254.
[4]*Ibid.*, Coll. Vol. I, Wiley, New York, 1941, p. 179.
[5]*Ibid.*, Coll. Vol. II, Wiley, New York, 1943, p. 379.
[6]B. C. Hesse, Am. Chem. J., *18*, 723 (1896).
[7]E. M. Gal and A. T. Shulgin, J. Am. Chem. Soc., *73*, 2938 (1951); Henry, Compt. rend., *102*, 1396 (1886).
[8]B. Lythgoe, A. R. Todd and A. Topham, J. Chem. Soc., *1944*, 315.
[9]E. L. Bennett, J. Am. Chem. Soc., *74*, 2420 (1952).
[10]J. Baddiley, B. Lythgoe and A. R. Todd, J. Chem. Soc., *1943*, 387.
[11]A. Pinner, Ber., *16*, 352, 1643 (1883).
[12]S. M. McElvain and J. W. Nelson, J. Am. Chem. Soc., *64*, 1825 (1942).

ADENINE-8-C^{14}

METHOD 1



(a)



(b)

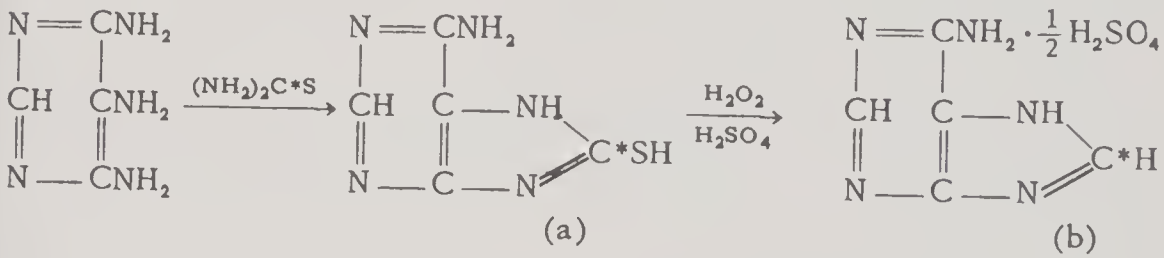
R. Abrams and L. Clark, J. Am. Chem. Soc., 73, 4609 (1951).

A. Procedure

(a) N-(4,6-*Diamino-5-pyrimidinyl)formamide-C*^{14} *Sulfate.* To a solution of 1.05 mmoles of sodium formate-C^{14} in 1.7 ml. of 0.07 *N* hydrochloric acid is added 1.05 mmoles of 4,5,6-triaminopyrimidine sulfate (Note 1). The mixture is heated to 50–70° and stirred until completely dissolved (Note 2). After 2 hours, the solution is stored in a refrigerator overnight. The product is centrifuged, washed with 0.2-ml. portions of alcohol and dried under vacuum; yield 77% (Note 3).

(b) *Adenine-8-C*^{14}. *N*-(4,6-Diamino-5-pyrimidinyl)formamide-C^{14} (1.09 mmoles) is suspended in 2.5 ml. of diethanolamine in an open tube and heated in a slow stream of nitrogen for 90 minutes at 210° (Note 4). The mixture is cooled, diluted with water, and treated with silver nitrate solution to precipitate the product as the silver salt,[1] which is centrifuged and washed. The salt is decomposed in *N* hydrochloric acid, and the filtrate is passed through a cation exchange column[1] (Note 5). The yield is 0.82 mmoles (75%), and the molar specific activity is 98% of that of the starting material (Note 6).

METHOD II



S. Graff, M. Engelman, H. B. Gillespie and A. M. Graff, Cancer Research, *11*, 388 (1951).

A. Procedure

(a) *6-Amino-8-purinethiol-8-C*^{14} (Note 7). An intimate mixture of 0.228 g. (3.0 mmoles) of thiourea-C^{14} and 0.385 g. (3.08 mmoles) of 4,5,6-triaminopyrimidine[6] is heated at 180° in a slow stream of nitrogen until the melt solidifies (30 min.). The product is dissolved in hot 2% ammonium hydroxide solution and decolorized with charcoal; then the filtrate is acidified with 6 *N* acetic acid and stored overnight in a refrigerator to crystallize; yield 0.224 g., 44% (Note 8).

(b) *Adenine-8-C*^{14} *Sulfate.* 6-Amino-8-purinethiol-8-C^{14} (0.200 g., 1.2 mmoles) is treated with 5 ml. of 3% hydrogen peroxide and heated in a boiling water-bath for 40 minutes. The solution is filtered, acidified with

dilute sulfuric acid and stored at 0° for 1.5 hours. After removal of the first crop of crystals (0.161 g.), the filtrate is treated with platinum-black to destroy the excess hydrogen peroxide, then filtered and concentrated to yield a small second crop. The combined crude product is decolorized with charcoal and recrystallized; yield 0.1742 g. (72%). The molar specific activity is 96.5% of that of the thiourea-C^{14} (Note 9).

B. Notes

1. Directions are outlined by Abrams for preparing the pyrimidine starting material.

2. The isotopic procedure of Clark and Kalckar[2] is modified by using the triaminopyrimidine sulfate salt which is less soluble than the hydrochloride.

3. Way and Mandel[3] have obtained a 95% yield by employing the free base, and a 30% excess of hydrochloric acid based on the formate. The ultraviolet absorption data were in agreement with those reported by Cavalieri.[4] Clark and Kalckar[2] obtained a 73% yield of *N*-(4,6-diamino-5-pyrimidinyl)formamide-C^{14} hydrochloride, radiochemical yield 95%.

4. The solvent boils at 270° (748 mm.), and the 200 × 8-mm. tube acts as an air condenser. Water evolution appears to cease within 1 hour. This solvent eliminates the exchange of formyl groups observed with *N*-formylmorpholine, see below.

5. The use of ion exchange purification avoids the use of decolorizing carbon, which is often accompanied by appreciable losses in very small scale preparations. A column of Nalcite-HCR, or Dowex-50, (250–500 mesh, 10 cm. × 1 cm.) is recommended. The operation of such columns has been described by Kunin.[5]

6. Way and Mandel[3] have obtained the hydrochloride hemihydrate in 65% yield. The purine fractions in the HCl eluate from a Dowex-50 cation exchange column were localized on a Beckman model DU quartz spectrophotometer at 260 mμ. The product was demonstrated[3] to be pure by chromatography and radioautography. The chromatogram on Whatman No. 1 paper was developed in 1-butanol saturated with 1% ammonium hydroxide, and in *t*-butyl alcohol-6 *N* hydrochloric acid-water (14 : 3 : 3v/v); R_f 0.49 and 0.24, respectively.

7. The method is adapted from one described by Johns.[6]

8. The compound is soluble in dilute alkali or mineral acids and is recrystallized from water. 6-Amino-8-purinethiol-8-C^{14} sulfate, $(C_5H_5N_5S)_2 \cdot H_2SO_4 \cdot 2H_2O$, separates upon cooling a hot solution of 0.143 g. of 6-amino-8-purinethiol-8-C^{14} in 14 ml. of 1 *N* sulfuric acid.

9. The compound crystallizes as $(C_5H_5N_5)_2 \cdot H_2SO_4 \cdot 2H_2O$. Cavalieri[7] states that recrystallization from 2 *N* sulfuric acid forms the hemihydrate, or the monohydrate if crystallization is rapid.

C. Other Preparations

N-(4,6-Diamino-5-pyrimidinyl)formamide-C^{14} hydrochloride and the C^{13}-sulfate have been prepared by a procedure similar to Method I in yields of 73% and 78%, respectively, by Clark[2] and Cavalieri.[7]

Adenine-8-C^{13} has been prepared in 70% yield by Cavalieri,[7] by heating *N*-(4,6-diamino-5-pyrimidinyl)formamide-C^{13} sulfate in a sealed tube with formamide; due to exchange, the specific activity was 25% of theoretical. Adenine-8-C^{14} hydrochloride has been prepared by Clark[2] (60% yield), by heating *N*-(4,6-diamino-5-pyrimidinyl)formamide-C^{14} hydrochloride in *N*-formylmorpholine; the specific activitv was 80% of theory. In three preparations at the 1-mmole scale by the same procedure, Gordon[8] has obtained 22.0–36.1% yields of adenine-8-C^{13} having a mole per cent excess isotope of 76–80% of the expected value. Abrams and Clark[9] have repeated this preparation with the sulfate (93% yield) and found the molar specific activity of the adenine-8-C^{14} to be only 51% of that of the starting material. They have studied this transformylation by preparing adenine-8-C^{14} by heating together various other solutes and solvents with the results outlined:

R-pyrimidine Sulfate	Solvent	Yield %	% relative specific activity
4,6-Diamino-5-formamido-	*N*-Formyl-C^{14}-morpholine	88	43,48
4,5,6-Triamino-	*N*-Formyl-C^{14}-morpholine	92	106
4,6-Diamino-5-formamido-C^{14}-	Morpholine : nitrobenzene	41	102
4,6-Diamino-5-formamido-C^{14}-	*N*-Formylmorpholine	93	51

Directions are given by Clark and Kalckar for preparing *N*-formyl-C^{14}-morpholine in 59% yield from formic-C^{14} acid. From the various methods of preparing adenine-8-C^{14} it is clear that no isotopic dilution occurs when only one source of formyl groups is present during cyclization.

The preparation of *N*-(4,6-diamino-5-pyrimidinyl)formamide-C^{14} hydrochloride, by the method of Clark and Kalckar,[2] has been reported by Abrams[10] and Griffin.[11]

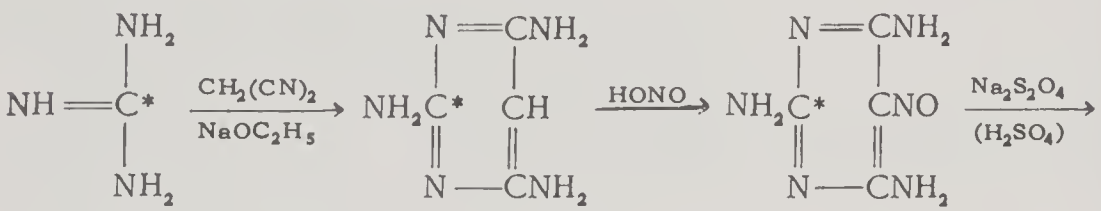
The preparation of adenine-8-C^{14} sulfate, by the method of Clark and Kalckar,[2] has been reported by Abrams[10] (yield 50% based on formate); Griffin[11] (yield 40% based on 4,5,6-triaminopyrimidine), Marrian[12] (radiochemical yield 50% based on formate) and Passonneau.[13]

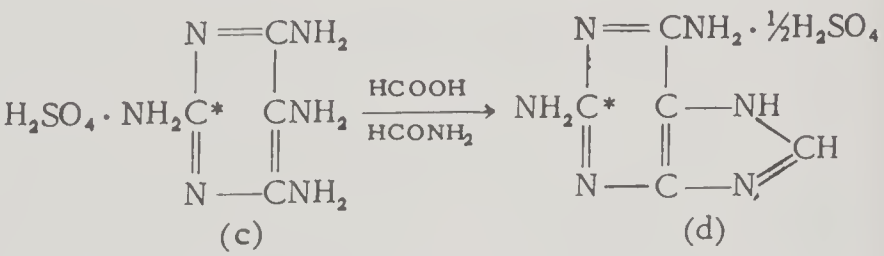
In a similar manner[2] from 2-methyl-4,5,6-triaminopyrimidine are prepared:[14] *N*-(4,6-diamino-2-methyl-5-pyrimidinyl)formamide-C^{14} hydrochloride [yield 80%, m.p. 267° (dec.), purity > 99%] and 2-methyladenine-8-C^{14} (yield 100%; molar specific activity 60% due to exchange, purity > 98%). The absorption maxima in 0.01 *N* hydrochloric acid are 265 mμ and 265-

266 mμ, respectively. Purity was established by paper chromatography of the two compounds with 2-propanol-ammonia and 2-propanol-hydrochloric acid,[15] respectively.

[1]R. Abrams, Arch. Biochem., *30*, 44 (1951).
[2]V. M. Clark and H. M. Kalckar, J. Chem. Soc., *1950*, 1029.
[3]J. L. Way and H. G. Mandel, private communication; J. L. Way, H. G. Mandel and P. K. Smith, Cancer Research, *14*, 812 (1954).
[4]L. F. Cavalieri and A. Bendich, J. Am. Chem. Soc., *72*, 2587 (1950).
[5]R. Kunin, Anal. Chem., *21*, 87 (1949).
[6]C. O. Johns, J. Biol. Chem., *21*, 319 (1915).
[7]L. F. Cavalieri and G. B. Brown, J. Am. Chem. Soc., *71*, 2246 (1949).
[8]M. Gordon, J. Chem. Soc., *1954*, 757.
[9]R. Abrams and L. Clark, J. Am. Chem. Soc., *73*, 4609 (1951).
[10]R. Abrams and J. M. Goldinger, Arch. Biochem. Biophys., *30*, 261 (1951).
[11]A. C. Griffin, W. E. Davis, Jr., and M. O. Tifft, Cancer Research, *12*, 707 (1952).
[12]D. H. Marrian, Biochem. et Biophys. Acta, *13*, 282 (1954).
[13]J. V. Passonneau and J. R. Tatter, Radiation Research, *3*, 304 (1955).
[14]D. H. Marrian, Biochem. et Biophys. Acta, *18*, 304 (1955).
[15]G. R. Wyatt, Biochem. J., *48*, 584 (1951).

2,6-DIAMINOPURINE-2-C^{14} SULFATE





L. L. Bennett, Jr., J. Am. Chem. Soc., 74, 2432 (1952).

A. Procedure

(a) *2,4,6-Triaminopyrimidine-2-C*^{14} (Note 1). To a solution of excess sodium ethoxide (8.7 mmoles) in absolute alcohol is added guanidine-C^{14} hydrochloride (6.25 mmoles) and an excess of malononitrile (6.8 mmoles). The stirred mixture is refluxed for 6 hours on a water-bath, then cooled,

and the precipitated product and sodium chloride are collected and washed with absolute alcohol.

(b) *2,4,6-Triamino-5-nitrosopyrimidine-2-C*^{14} (Note 1). To a stirred solution of the 2,4,6-triaminopyrimidine-2-C^{14} in dilute acetic acid is added an aqueous solution of sodium nitrite (6.4 mmoles). The bright red precipitate is filtered off as quickly as possible and washed with water to avoid continued contact with excess nitrous acid.

(c) *2,4,5,6-Tetraaminopyrimidine-2-C*^{14} *Sulfate* (Note 1). To a stirred aqueous suspension of the 2,4,6-triamino-5-nitrosopyrimidine-2-C^{14} at 60–70° is added slowly solid sodium hydrosulfite until the pink color disappears (50 mmoles). The mixture is acidified with sulfuric acid and filtered at the boiling point, and the filtrate is stored in a refrigerator to crystallize; yield 30–45% based on guanidine.

(d) *2,6-Diaminopurine-2-C*^{14} *Sulfate* (Notes 2 and 3). 2,4,5,6-Tetraaminopyrimidine-2-C^{14} sulfate (1.33 g.) is heated for 2.5 hours at 160° in a sealed tube with formamide (15 ml.) and 98% formic acid (0.4 ml.). The crystals deposited upon cooling are twice recrystallized from 2 *N* sulfuric acid; yield 65–85%. Filter paper chromatography shows the presence of a trace of labeled guanine as the only contaminant (Note 4).

B. Notes

1. The procedure is an adaptation of Mallette's modification of the method of Traube.[1] For a similar preparation see 2,5,6-triamino-4-pyrimidinol-6-C^{14} sulfate.

2. The composition is $(C_5H_6N_6)_2 \cdot H_2SO_4 \cdot H_2O$. The anhydrous compound is formed by drying over phosphorus pentoxide for 4 hours at 140° and 1 mm.

3. The procedure is that described by Bendich.[2]

4. Bendich[2] found that the product prepared in this manner was homogeneous, when examined by countercurrent distribution in butanol-1 *M* potassium phosphate buffer (pH 6.5), K = 1.21. The product is homogeneous when examined by filter paper chromatography,[3] using an ascending solvent system of 1-butanol-diethylene glycol-water (4:1:1) in an ammonia atmosphere; R_f 0.33.

C. Other Preparations

2,6-Diaminopurine-2-C^{13} sulfate has been prepared by Bendich[4] from guanidine-C^{13} nitrate by the described procedure. The last step conversion was in 88% yield. After drying under vacuum at room temperature over phosphorus pentoxide, the composition of the product is $C_5H_6N_6 \cdot 1/2\,H_2SO_4 \cdot 1/2\,H_2O$.

The preparation of 2,4,5,6-tetraaminopyrimidine-2-C^{14} in 25% yield has been reported by Skipper.[5] Guanidine-C^{14} hydrochloride was condensed

with phenylazomalononitrile in butanol, and the azo linkage was reduced to the amine with zinc dust (see 4,5,6-triaminopyrimidine-4-C^{14} sulfate).

[1]M. F. Mallette, E. C. Taylor, Jr., and C. K. Cain, J. Am. Chem. Soc., *69*, 1814 (1947); W. Traube, Ber., *37*, 4544 (1904).

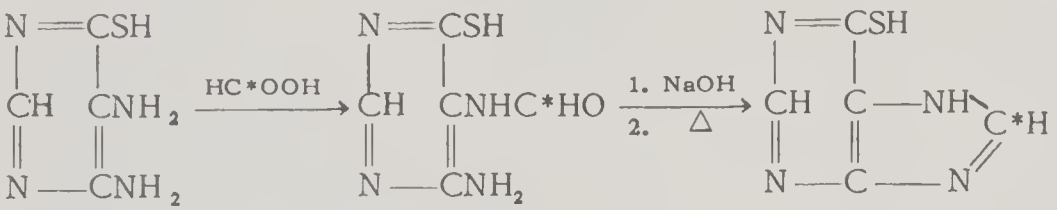
[2]A. Bendich, J. F. Tinker and G. B. Brown, J. Am. Chem. Soc., *70*, 3109 (1948).

[3]E. Vischer and E. Chargaff, J. Biol. Chem., *176*, 703 (1948).

[4]A. Bendich, S. S. Furst and G. B. Brown, J. Biol. Chem., *185*, 423 (1950).

[5]H. Skipper and L. L. Bennett, Nucleonics, 7 (4), 50 (1950).

6-PURINETHIOL-8-C^{14}
(6-Mercaptopurine-8-C^{14})



G. B. Elion and G. H. Hitchings, J. Am. Chem. Soc., *76*, 4027 (1954).

A. Procedure

A mixture of 0.1915 mmole of sodium formate-C^{14}, 0.5 g. (3.5 mmoles) of powdered 5,6-diamino-4-pyrimidinethiol (Note 1) and 2.2 ml. of 90% formic-C^{14} acid is heated at 85° for 4–5 hours. The mixture is evaporated at 50° under reduced pressure, the last traces of formic-C^{14} acid being removed by evaporation of three 3-ml. portions of added water. To the residue, *N*-(6-amino-4-mercapto-5-pyrimidinyl)formamide-C^{14}, is added 3.5 ml. of *N* sodium hydroxide, and the mixture is evaporated at 50° under reduced pressure. The residual sodium salt is heated slowly to 220°, and the temperature is maintained at 220–240° for 1 hour. The residue is dissolved in 70 ml. of water, and the filtered solution is adjusted to pH 5 with acetic acid and filtered hot. The filtrate is stored in a refrigerator for 24 hours; then the product is collected, washed with water and dried in a vacuum desiccator; yield 0.38 g., 64% (Note 2). The ultraviolet absorption spectrum is the same as that of an authentic pure sample.

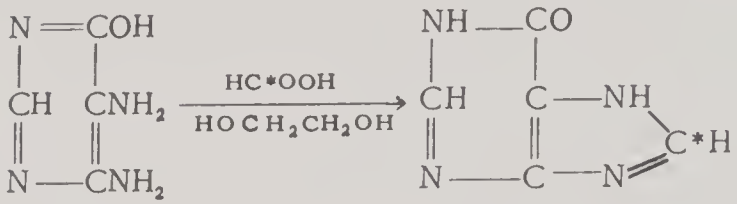
B. Notes

1. Directions are given by Elion for preparing the starting material from 4-amino-6-chloro-5-nitropyrimidine[1] by reduction with potassium hydrosulfide and treatment with hydrogen sulfide.

2. The monohydrate, m.p. 313–314° (dec.), loses water of crystallization at 140°, but not at 110°.

[1]W. R. Boon, W. G. M. Jones and G. R. Ramage, J. Chem. Soc., *1951*, 96.

HYPOXANTHINE-8-C^{14}
[6(1*H*)-Purinone-8-C^{14}]



J. A. Montgomery, Southern Research Institute, Birmingham, Alabama, private communication.

A. Procedure (Note 1)

To a concentrated aqueous solution of 0.272 g. (4 mmoles) of sodium formate-C^{14} in a short test tube is added a slight excess of 6 *N* hydrochloric acid, followed by 0.510 g. (4.04 mmoles) of 5,6-diamino-4-pyrimidinol and 8 ml. of ethylene glycol. The stirred mixture is heated to 80° for 1 hour, then gradually to 220°, where it is held for another hour. The mixture is stored for 2 days in a refrigerator; then the product is filtered off, dissolved in dilute ammonia, and decolorized with charcoal, and the ammonia is boiled off. The precipitated product, a cream-colored powder, is filtered off and dried; yield 0.297 g. (54.3%). The molar specific activity is in agreement with that of the starting material. Paper chromatography (Note 2) and radioautography indicate that 99% of the radioactivity is found in the product (Note 3).

B. Notes

1. The procedure is a modification of the method of Weygand and Grosskinsky for the preparation of guanine-8-C^{14}.

2. A paper chromatogram is made using an ascending solvent system of 5% disodium phosphate-isopentyl alcohol, as described by Carter[1] for separating purine and pyrimidine bases.

3. The enzymatic oxidation of hypoxanthine-2-C^{14}, -4-C^{14} and -8-C^{14} to uric-2-C^{14}, -4-C^{14} and -8-C^{14} acid, respectively, is described by Brandenberger.[2]

4. The chloride form of the resin is used, 200–400 mesh, 15 × 210 mm.

C. Other Preparations

Xanthine-8-C^{14}, [2,6(1*H*,3*H*)-purinedione-8-C^{14}], is prepared in a similar manner. A mixture of sodium formate-C^{14} (1.19 mmoles) and 5,6-diamino-2,4-pyrimidinediol is stirred overnight before heating and is not stored in the refrigerator for 2 days afterward. The crude product is purified by dissolution in ammonium hydroxide and adsorption on a column of Dowex-1 (Note 4), which is then washed with 2 l. of water and eluted with an

0.05 *M* ammonium chloride — 4 *N* ammonium hydroxide solution. The product begins to appear in the filtrate after 600 ml. of solution has passed through and is completely eluted by an additional 1500 ml. The eluate is concentrated to small volume, and the precipitate is collected. Purification is effected by dissolution in 1:1 ammonium hydroxide, filtration, and reprecipitation with acetic acid. The colorless powder is collected, washed with alcohol and ether, and dried in a vacuum desiccator; yield 0.213 g. (52.7%). Paper chromatography and radioautography, as described for hypoxanthine-8-C^{14}, indicate that the purity is higher than 99%.

The preparation of hypoxanthine-8-C^{14}, essentially by the method described, has been reported by Weygand.[3]

The preparation of hypoxanthine-8-C^{14} by the deamination of adenine-8-C^{14} with nitrous acid has been described by Brandenberger.[2]

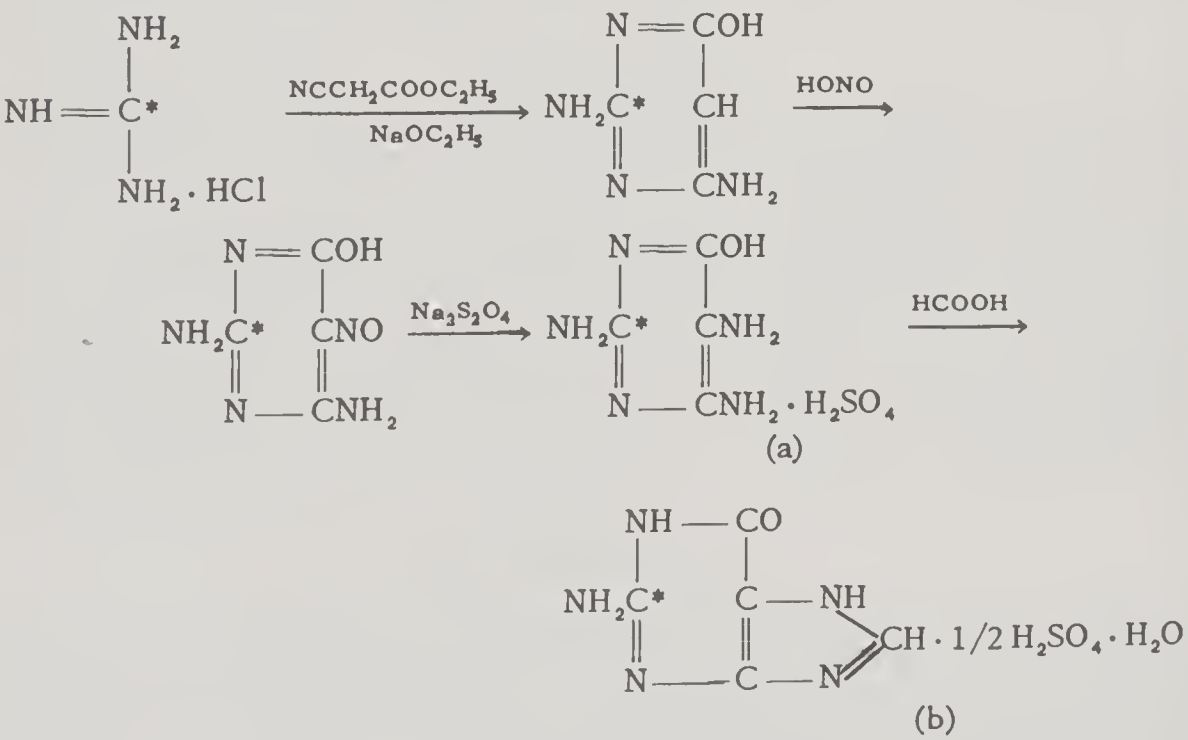
The preparation of xanthine-8-C^{14} (60% yield) by a similar deamination of guanine-8-C^{14} has been described by Weygand.[3]

[1]C. E. Carter, J. Am. Chem. Soc., *72*, 1468 (1950).

[2]H. Brandenberger, Biochim. et Biophys. Acta, *15*, 108 (1954).

[3]F. Weygand, H. Klebe, A. Trebst and H. Simon, Z. Naturforsch., *9b*, 450 (1954).

GUANINE-2-C^{14} SULFATE
(2-Aminohypoxanthine-2-C^{14} Sulfate)



L. L. Bennett, Jr., J. Am. Chem. Soc., *74*, 2432 (1952); Cancer Research, *10*, 644 (1950); Nucleonics, 7 (4), 49 (1950).

A. Procedure (Note 1)

(a) *2,5,6-Triamino-4-pyrimidinol-2-C^{14} Sulfate* (Note 2). A mixture of guanidine-C^{14} hydrochloride, ethyl cyanoacetate and excess sodium ethoxide in absolute alcohol is refluxed for 3 hours. The mixture is diluted with water and acidified with hydrochloric acid. The resulting solution of 2,6-diamino-4-pyrimidinol-2-C^{14} is nitrosated by slow addition of sodium nitrite solution. After the mixture is heated to boiling, it is allowed to cool, and the rose-colored 2,6-diamino-5-nitroso-4-pyrimidinol-2-C^{14} crystallizes. Reduction of the nitroso group is effected (Note 3) by making the mixture basic with sodium hydroxide and adding sodium hydrosulfite to the vigorously stirred mixture at 70–80° during 15 minutes. Heating and stirring are continued for 30 minutes; then the mixture is filtered at the boiling point and cooled to 0–10° to crystallize the product as the sulfate monohydrate; yield 67–74% (Note 4).

(b) *Guanine-2-C^{14} Sulfate.* The 2,5,6-triamino-4-pyrimidinol-2-C^{14} sulfate is converted to guanine-2-C^{14} by heating with 98–100% formic acid after the method of Traube.[1] The yield of product, isolated as the sulfate dihydrate, is 85% after one crystallization (Note 5). Filter paper chromatography shows the presence of only one active compound (Note 6).

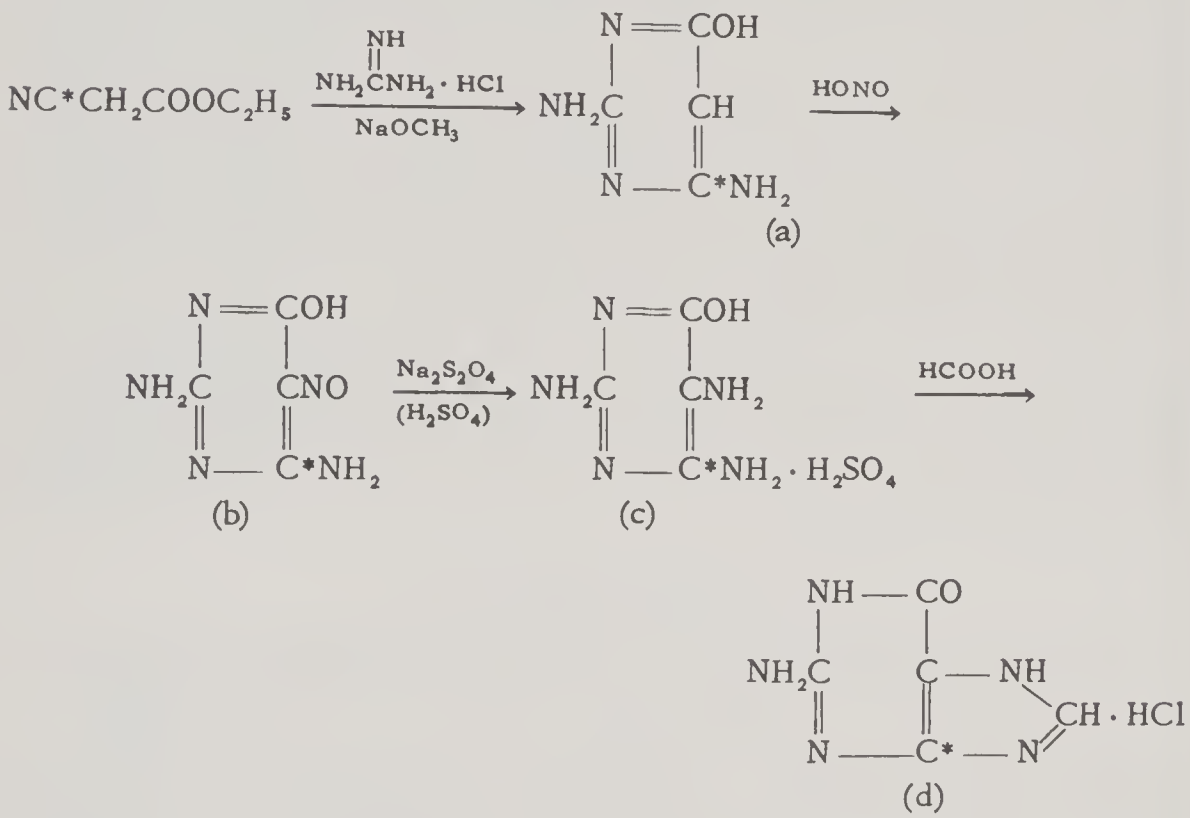
B. Notes

1. See guanine-4-C^{14} hydrochloride for details of a similar preparation.
2. The procedure is essentially that of Cain, Mallette and Taylor.[2]
3. The yield is not lowered when the reduction is carried out without isolating the intermediate. Petering and Schmitt[3] have achieved the reduction with zinc and hydrochloric acid. The sulfate[1,3] is prepared from a solution of either the free base or dihydrochloride by the addition of dilute sulfuric acid.
4. The product may be purified by crystallization from two parts of water. Yields of 87% have been peported for preparations at the 1-mole scale.[2] Similar directions are given by Weygand[4] for the isotopic preparation of the sulfate dihydrate in 55% yield based on barium carbonate-C^{14} at the 1-mmole level. A 90% yield based on guanidine-C^{14}, by a similar procedure, has been reported by Fong.[5]
5. The product may be recrystallized from 2 *N* sulfuric acid with 85–90% recovery. The anhydrous compound is obtained by drying at 140–160° (1 mm.) over phosphorus pentoxide.
6. An ascending filter paper chromatogram, on Whatman No. 1 paper, is developed in a solvent system of 1-butanol-diethylene glycol-water (R:1:1) in an ammonia atmosphere; R_f 0.21. The spot is visible in ultraviolet light.

[1]W. Traube, Ber., 33, 1371 (1900).
[2]C. K. Cain, M. F. Mallette and E. C. Taylor, Jr., J. Am. Chem. Soc., 68, 1996 (1946).

[3]H. G. Petering and J. A. Schmitt, J. Am. Chem. Soc., *71*, 3977 (1949).
[4]F. Weygand, H. J. Mann and H. Simon, Ber., *85*, 463 (1952).
[5]C. T. Fong, C. M. Judson, N. H. Marsh and D. J. Salley, Nucleonics, 7 (4), 49 (1950).

GUANINE-4-C^{14} HYDROCHLORIDE



E. L. Bennett, J. Am. Chem. Soc., *74*, 2420 (1952); Nucleonics, 7 (4), 49, 50 (1950).

A. Procedure (Note 1)

(a) *2,6-Diamino-4-pyrimidinol-6-C^{14}.* To 18 mmoles of ethyl cyano-C^{14}-acetate is added a solution prepared from 4.0 g. of guanidine hydrochloride and 1.04 g. of sodium in 20 ml. of absolute methanol (Note 2). The mixture is refluxed for 8 hours under anhydrous conditions; then the solvent is removed under reduced pressure, yielding a heavy brown syrup (Note 3).

(b) *2,6-Diamino-5-nitroso-4-pyrimidinol-6-C^{14}.* To the crude 2,6-diamino-4-pyrimidinol-6-C^{14} dissolved in 40 ml. of water is added 30 ml. of 2 *M* sodium acetate, 8 ml. of 6 *N* hydrochloric acid and an excess of sodium nitrite (Note 4). After several minutes, 0.67 ml. of additional hydrochloric acid is added, and the solution is stored for several hours

at 0°. The rose-colored product is filtered off and washed with water (Note 5).

(c) *2,5,6-Triamino-4-pyrimidinol-6-C^{14} Sulfate.* The 2,6-diamino-5-nitroso-4-pyrimidinol-6-C^{14} is mixed with 46 ml. of 0.5 *N* sodium hydroxide. After several minutes, 2.5 ml. of 9 *N* sulfuric acid is added with vigorous stirring; the solution is warmed to 50°, and 10 g. of sodium hydrosulfite is added in portions during 15 minutes. The warm solution is filtered, acidified with sulfuric acid and cooled to 0° to crystallize the product; yield 2.65 g., 52% based on sodium cyanide (Note 6). By working up the mother liquor with 0.215 g. of carrier, 0.338 g. of additional product is obtained with a specific activity equivalent to 3% radiochemical yield.

(d) *Guanine-4-C^{14} Hydrochloride.* A mixture of 1.29 g. of the C^{14}-pyrimidinol sulfate, 0.357 g. of sodium formate and 15 ml. of 98–100% formic acid is refluxed for 15 hours. The solvent is evaporated under reduced pressure, and the residue is dissolved in 25 ml. of 0.5 *N* hydrochloric acid, decolorized with Norit and stored at 5° for several days. The product is collected, washed with cold water, and air-dried; the yield of dihydrate is 0.924 g. (83%), 45% from sodium cyanide (Note 7). Paper chromatography shows the presence of only one radioactive compound (Note 8).

B. Notes

1. The procedure is a modification of the method of Traube,[1] essentially as described by Cain, Mallette and Taylor.[2] See guanine-2-C^{14} sulfate for a similar preparation.

2. Yields are similar, but more variable, when ethanol or butanol are used.

3. The yield is 60%[1] to 80%[3]. The sulfate may be crystallized from diluted sulfuric acid at 0°.

4. The pH should be more acid than 5, at the start. The addition of the nitrite to the pyrimidine is preferable[3] to the converse reaction.

5. The product is more conveniently used in the next step if it is not dried. The yield is 95%.[3]

6. Mandel[3] has reported a yield of 83% by reduction with ammonium sulfide.

7. Cain[2] has reported a yield of 87% based on guanidine hydrochloride at the 1.05-mole scale. Mandel[4] has reported an 81% yield of the free base at the 19-mmole scale.

8. The free base is formed[4] by filtering a hot acid solution into an excess of dilute ammonia at 0°. A chromatogram, in a system of 1-butanol and water (86:14 v/v), indicates the presence of only one radioactive compound. A countercurrent distribution in a system of *M* phos-

phate buffer (pH 6.46) and 1-butanol indicates that the product is homogeneous, and agrees in partition coefficient (2.2) and spectral properties with the values reported in the literature. The molar specific activity is 96.5% of that of the 2,5,6-triamino-4-pyrimidinol-6-C^{14} sulfate. A degradation is reported by Elwyn.[5]

C. Other Preparations

Cavalieri[6] has prepared guanine-4-C^{13} from methyl cyano-C^{13}-acetate by a similar procedure.

Mandel[3,4] has reported the preparation of 2,5,6-triamino-4-pyrimidinol-6-C^{14} sulfate (yield 65% based on ethyl cyano-C^{13}-acetate) essentially by the described method. The ring closure was effected by a modification of this method, forming guanine-4-C^{14} in 45% yield based on sodium cyanide-C^{14}.

[1]W. Traube, Ber., *33*, 1371 (1900); W. Traube and H. W. Dudley, Ber., *46*, 3839 (1913).

[2]C. K. Cain, M. F. Mallette and E. C. Taylor, Jr., J. Am. Chem. Soc., *68*, 1996 (1946).

[3]H. G. Mandel, E. L. Alpen, W. D. Winters and P. K. Smith, J. Biol. Chem., *193*, 63 (1951); private communication.

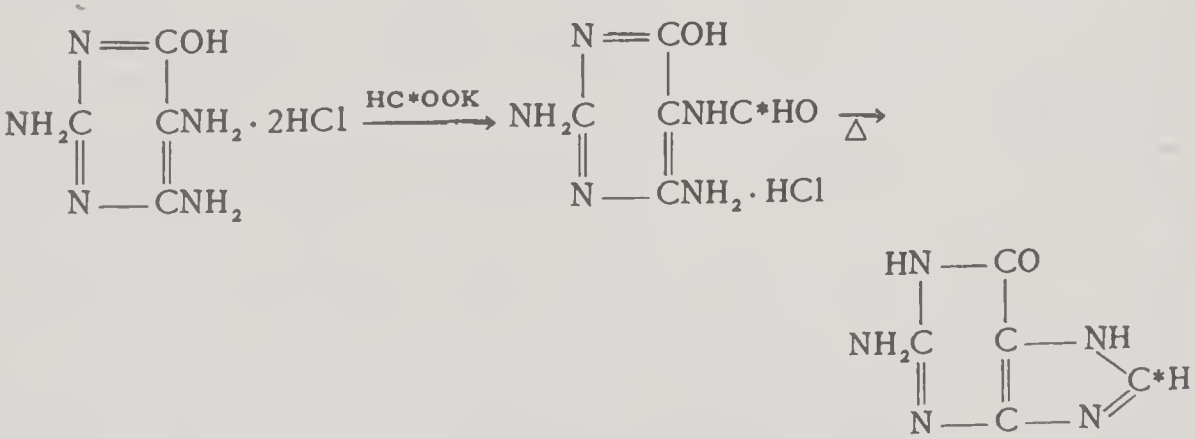
[4]H. G. Mandel and P. E. Carlo, J. Biol. Chem., *201*, 335 (1953); private communication.

[5]D. Elwyn and D. B. Sprinson, J. Biol. Chem., *207*, 467 (1954).

[6]L. F. Cavalieri, J. F. Tinker and G. B. Brown, J. Am. Chem. Soc., *71*, 3973 (1949).

GUANINE-8-C^{14}

METHOD I

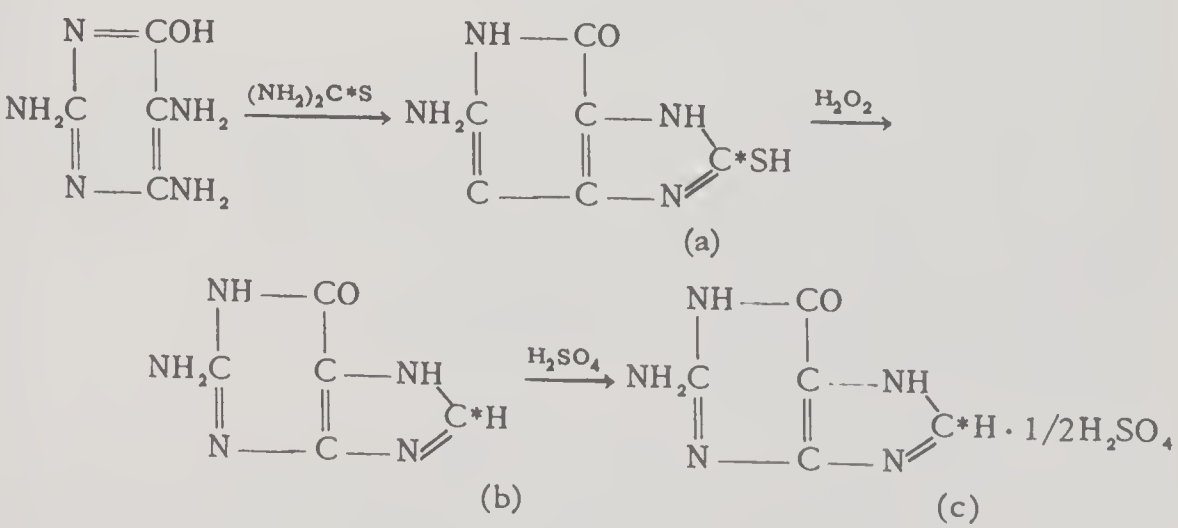


F. Weygand and O. A. Grosskinsky, Ber., *84*, 839 (1951).

A. Procedure (Note 1)

A mixture of 0.4 ml. of aqueous solution containing 0.925 mmole of potassium formate-C^{14} (Note 2), 0.232 g. (1 mmole) of pulverized 2,5,6-triamino-4-pyrimidinol dihydrochloride monohydrate and 2 ml. of ethylene glycol is stirred overnight at room temperature in a test tube. The stirred mixture is heated for 45 minutes at 70°; then the temperature is raised to 200° over a period of 30 minutes and held there for 2 hours while water and any formic acid are distilled. The mixture is transferred with ethylene glycol to a distillation flask, and the solvent is removed by vacuum distillation. The residue is boiled with 20–30 ml. of water, made alkaline with a few drops of ammonium hydroxide, then concentrated until the solution tests neutral, and stored in a refrigerator for a day to crystallize. The crude product is filtered off and dried in a desiccator; yield 0.120–0.130 g., 75–80% (Note 3). Purification is effected by dissolving the crude product in 50 ml. of hot 2 *N* hydrochloric acid, storing for 2 days at room temperature and filtering off a precipitated, colored impurity. The filtrate is evaporated to dryness under vacuum over sulfuric acid and potassium hydroxide. The residue is treated with excess hydrochloric acid and evaporated to dryness. The yield of guanine-8-C^{14} hydrochloride is 0.166 g. (Note 4). Further purification is effected by converting the salt to the free base by dissolving in half-concentrated warm ammonia solution and boiling until the product precipitates. The mixture is stored for 1 day in a refrigerator; then the product is collected and washed with a little water. The yield of spectroscopically pure product is 0.105–0.114 g., 70–75% based on reacted carbon dioxide. The molar specific activity is 98–99% of that of the starting material.

METHOD II



S. Graff, M. Engelman, H. B. Gillespie and A. M. Graff, Cancer Research, *11*, 388 (1951).

A. Procedure

(a) *2-Amino-8-purinethiol-8-C*^{14} (Note 5). An intimate mixture of 0.248 g. (3.26 mmoles) of thiourea-C^{14} and 0.460 g. (3.26 mmoles) of 2,5,6-triamino-4-pyrimidinol is heated at 180° for 1 hour in a slow stream of nitrogen (Note 6). The product is dissolved in hot dilute alkali, and the residue is filtered off and washed with hot water. The combined filtrate is acidified with 3 *N* acetic acid and cooled, and the product is collected by centrifuging, washed with water, alcohol, and ether, then vacuum-dried over phosphorus pentoxide. The yield of crude product is 0.464 g. (77.8%).

(b) *Guanine-8-C*^{14}. A mixture of 0.460 g. of the thiol and 10 ml. of 3% hydrogen peroxide is heated in a boiling water-bath for 30 minutes, then treated with an additional 2 ml. of hydrogen peroxide, and the heating is continued for 30 minutes. The mixture is brought to boiling and treated with 1 ml. of 10 *N* sulfuric acid, and the insoluble residue is filtered off and washed with 0.1 *N* sulfuric acid and water. The hot combined filtrate (75 ml.) is made alkaline with concentrated ammonia to precipitate the crude product; yield 0.294 g. (77.5%).

(c) *Guanine-8-C*^{14} *Sulfate.* The crude guanine-8-C^{14} is refluxed with 30–35 ml. of 0.2 *N* sulfuric acid and a small amount of charcoal for 10 hours. The insoluble material is filtered off from the hot solution and washed with 0.2 *N* sulfuric acid. The combined filtrate is cooled to crystallize the pure salt; yield 0.304 g.

B. Notes

1. The method is essentially that developed by Clark and Kalckar for preparing adenine-8-C^{14} (Method I).
2. The solution must be sulfate-free.
3. The purity is 90–95%.
4. The salt contains 1.5 molecules of water of crystallization and a trivial amount of nonpurine impurity, but is sufficiently pure for many uses. The phosphotungstic acid test[1,2] may be used to indicate the presence of pyrimidine starting material or intermediate. The chromatography of guanine is described by Heinrich.[2,3]
5. The method is adapted from one described by Johns.[4]
6. The melt resolidifies in about 30 minutes.

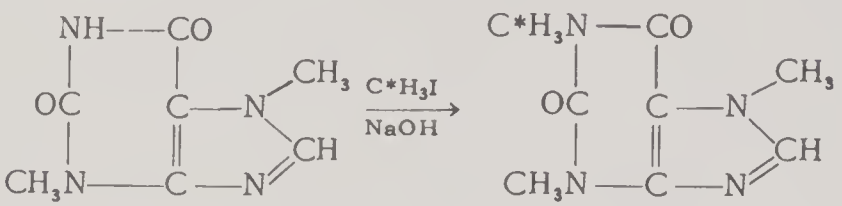
C. Other Preparations

The preparation of guanine-8-C^{14}, by the method of Clark and Kalckar for preparing adenine-8-C^{14}, has been described by Balis[5] (yield 79%, radiochemical yield 40%), and Abrams[6] (yield 50%, radiochemical yield about 18%). The use of a modification of Method I has been described by

Heinrich[4] (yield over 54% based on 2,5,6-triamino-4-pyrimidinol dihydrochloride at the 2.2-mmole level).

[1]H. B. Lewis and B. H. Nicolet, J. Biol. Chem., *16*, 369 (1914).
[2]M. R. Heinrich, V. C. Dewey and G. W. Kidder, J. Am. Chem. Soc., *75*, 1741 (1953); W. Traube, Ber., *33*, 1371 (1900).
[3]M. R. Heinrich, V. C. Dewey, R. E. Parks, Jr., and G. W. Kidder, J. Biol. Chem., *197*, 199 (1952).
[4]C. O. Johns, J. Biol. Chem., *21*, 319 (1915).
[5]M. E. Balis, G. B. Brown, G. B. Elion, G. H. Hitchings and H. Vanderwerff, J. Biol. Chem., *188*, 217 (1951).
[6]R. Abrams and J. M. Goldinger, Arch. Biochem. Biophys., *30*, 261 (1951).

1-METHYL-C^{14}-3,7-DIMETHYLXANTHINE (C_1^{14}-Caffeine)



A. Murray III and A. R. Ronzio, Atomic Energy Commission Report, AECU-2838; Nuc. Sci. Abstracts, *8*, 3313 (1954).

A. Procedure (Note 1)

The apparatus consists of a water-jacketed, heavy-walled bomb tube equipped with a glass-enclosed, magnetic stirring bar, a wide-bore pressure stopcock and attachment to a vacuum manifold (Note 2). To a frozen solution of 0.399 g. (2.22 mmoles) of theobromine, 0.0930 g. (2.33 mmoles) of sodium hydroxide (5% excess) and approximately 4 ml. of water (Note 3) is added 0.2865 g. (2.02 mmoles) of methyl-C^{14} iodide by vacuum distillation, with liquid nitrogen cooling. The system is restored to atmospheric pressure, the tube is closed, and the stirred mixture is heated under reflux at 80° for 2 hours (Note 4). The mixture is frozen, the tube is evacuated, 0.08 g. (0.56 mmole) of nonisotopic methyl iodide is added, and the reaction is continued for 1.5 hours (Note 5). The mixture is washed out of the tube with water and evaporated to dryness under a warm air stream. The residue is extracted with three portions of chloroform (total 40 ml.), and the filtered solution is evaporated to dryness; yield 0.4287 g. (99.5%) of crude product (Note 6). Purification is achieved by vacuum sublimation at 90–120° (10 μ), followed by two recrystallizations from 7–8 ml. of toluene at −10° to −15°; yield 0.372 g., m.p. 229–230°, 86.7% based on theobromine (Note 7).

B. Notes

1. The procedure is adapted from the method of Fischer.[1] Attempts to methylate theobromine with methyl iodide in nonaqueous media, see estrone methyl-C^{14} ether, were unsuccessful.

2. The apparatus is that used in the preparation of methyl-C^{14} iodide, Method II.

3. The sodium hydroxide is added accurately as a suitable aliquot of a standard solution.

4. The refluxing ceases by 30 minutes.

5. The total methyl iodide is in excess over the theobromine, which is in 10% excess over the isotopic methyl iodide.

6. A comparable trial preparation melted at 225–230° in a sealed capillary (remelted, 227–229°). The solubility[2] per 100 g. of chloroform is: caffeine 11.8 g. (18°), and theobromine 0.060 g. (15.5°).

7. A purified authentic sample melts at 230–231°. A synthesis was developed[3] for labeling with methyl-C* *p*-toluenesulfonate as the methylating agent, by a modification of the method of Rodionow[4] for alkylating amines and phenols. The yield of crude caffeine was 75–83% based on methanol (2.5 mmoles) in five trial runs without purification of the intermediate. Excess sodium hydroxide interferes.

Methyl-C* *p*-toluenesulfonate was prepared[3] by a modification of the method of Roos, Gilman and Beaber.[5] To an ice-cooled, stirred solution of methanol-C* and a 10% excess of purified tosyl chloride[4] in acetone was added a stoichiometric amount of 9.4 *N* sodium hydroxide by microburet. The mixture was stirred for 4 hours, without replenishment of the ice-bath, then was diluted with 25 ml. of water, basified with sodium bicarbonate and extracted continuously with ether for 6 hours. The solvent was removed by evaporation; yield 94–97% at the 2.4–5.0-mmole scale. The crude product was crystalline while stored in a refrigerator.

C. Other Preparations

By the same procedure, starting with theophylline, is prepared 7-methyl-C^{14}-1,3-dimethylxanthine; crude yield 98.8%, purified yield 76%, m.p. 230–231°.

[1]E. Fischer and F. Ach, Ber., *31*, 1980 (1898).

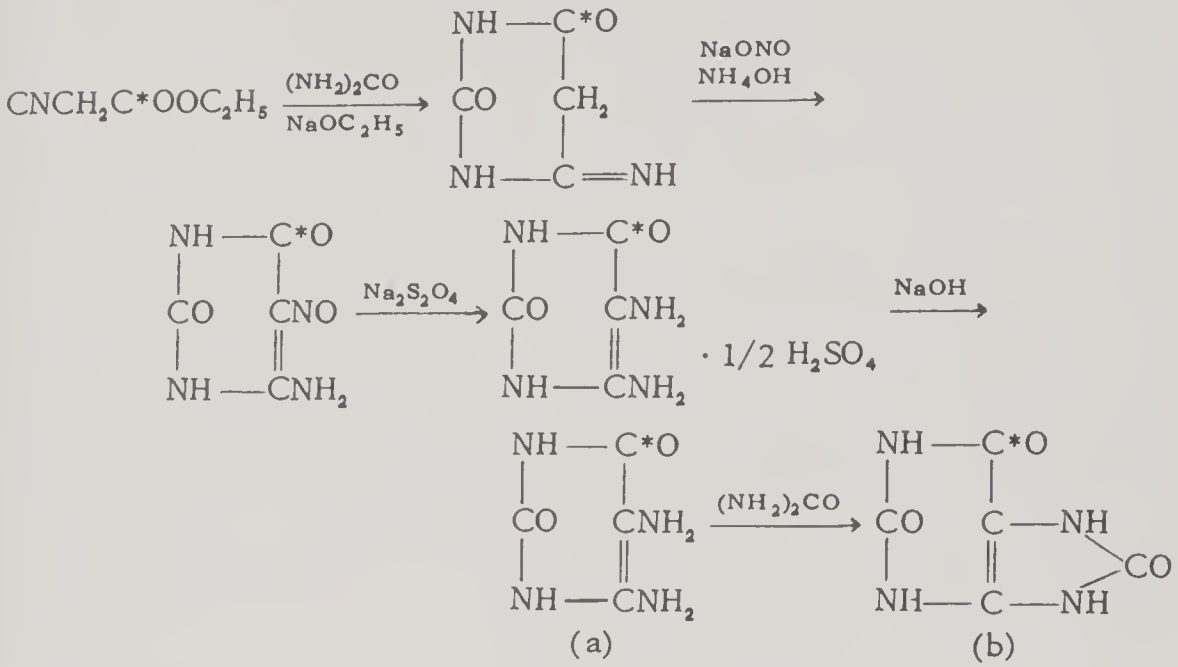
[2]C. S. Marvel, F. C. Dietz and M. J. Copley, J. Am. Chem. Soc., *62*, 2273 (1940).

[3]A. Murray III, unpublished work.

[4]W. Rodionow, Bull. soc. chim., France, (4) *39*, 322 (1926).

[5]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 145.

URIC-6-C^{14} ACID
[2,6,8(1*H*,3*H*,9*H*)-Purinetrione-6-C^{14}]



R. Bentley and A. Neuberger, Biochem. J., *52*, 694 (1952).

A. Procedure

(a) *5,6-Diaminouracil-4-C^{14}*, [*5,6-Diamino-2,4(1H,3H)-pyrimidinedione-4-C^{14}*]. Ethyl cyanoacetate-1-C^{14} (10.74 g., b.p. 97° at 16 mm.) is condensed with urea, and the 6-aminouracil-4-C^{14} then is nitrosated and reduced according to the procedure of Bogert and Davidson,[1] which follows (Note 1); yield 8 g., 74%.

To a solution of 4.6 g. of sodium in 75 ml. of absolute alcohol is added 6 g. of urea and 11.3 g. of ethyl cyanoacetate. The mixture is refluxed for 2 hours, then filtered hot, and the sodium salt of 6-aminouracil is washed with alcohol and air dried (Note 2). To a solution of the salt in 100 ml. of water is added 50 g. of ice and 8 g. of sodium nitrite; then the mixture is added to a slush of 24 g. of acetic acid, 25 ml. of water and 75 g. of ice. The mixture is made basic with concentrated ammonia, and the salt of 6-amino-5-nitrosouracil[-4-C^{14}] is collected. To a suspension of the salt in 250 ml. of hot water is added 40 g. of sodium hydrosulfite (technical), and the rapidly stirred mixture is heated to boiling (Note 3). The mixture is cooled, and the crude 5,6-diaminouracil[-4-C^{14}] sulfate is collected. Purification is accomplished by dissolution in 100 ml. of 6% sodium hydroxide solution containing a little sulfite, and addition of the filtered solution to a boiling solution of 10 ml. of concentrated sulfuric acid in 100 ml. of water, followed by cooling, washing of the salt with water and drying at 120°; yield 62%.

The free pyrimidine base is prepared from the sulfate according to the procedure of Johnson and Johns,[2] which follows. A thin paste of the pulverized salt and water is neutralized by trituration with the calculated amount of *N* sodium hydroxide. The product is filtered off, washed with water, alcohol, and ether, then dried at 75° (Note 4).

(b) *Uric-6-C*^{14} *Acid* (Note 5). A finely ground mixture of 4 g. of 5,6-diaminouracil-4-C^{14} and 4 g. of urea is heated for 1 hour in an oil-bath at 160°. The solid (Note 6) is dissolved in 0.5 *N* potassium hydroxide, and the filtered solution is acidified with concentrated hydrochloric acid. The crude product is collected and dissolved in 0.5 *N* piperidine, and the solution is treated with an excess of solid ammonium chloride and stored at 2° for 24 hours. The ammonium urate-6-C^{14} is collected and digested with 2 *N* hydrochloric acid at 70°. The still-impure product is dissolved in concentrated sulfuric acid, and the filtered solution is carefully treated with water to precipitate the product. Purification is accomplished by recrystallization from a large volume of boiling water (Note 7).

B. Notes

1. The procedure is a simplification of the method of Traube.[3]

2. 6-Aminouracil may be purified by dissolution in water and reprecipitation with acetic acid.

3. The rose-colored salt is converted to the insoluble buff-colored 5,6-diaminouracil sulfate.

4. Recrystallization from water results in partial decomposition.

5. The procedure is adapted from the method of Johnson and Johns.[2] An improved procedure, without the necessity for isolating the free 5,6-diaminouracil-4-C^{14} is described by Dalgliesh[4] (see Other Preparations); the product is cleaner and spectroscopically pure.

6. The mixture liquifies, then resolidifies.

7. The uric acid is characterized by elementary analysis, ultraviolet absorption,[5] and enzymatic oxidation with uricase to carbon-C^{14} dioxide.

8. Degradations are described by Sonne,[6] Dalgliesh[4] and Brandenberger (see potassium allantoxanate-2-C^{14}). Calculation based on the reported data indicates that the molar specific activity is about 6% of that of the starting material.

C. Other Preparations

The preparation of uric-6-C^{14} acid by a modification of this method employing methyl cyanoacetate-1-C^{14}, has been reported by Brandenberger.[7]

The preparation of uric-4-C^{14} acid by an improvement of this method has been described by Dalgliesh.[4] 5,6-Diaminouracil-6-C^{14} sulfate (4.8

g.), prepared in turn from ethyl cyano-C^{14}-acetate, 6-aminouracil-6-C^{14} and 6-amino-5-nitrosouracil-6-C^{14} by the described procedure (yield 57%), is powdered with 14 g. of urea, and the mixture is heated at 180° for 1 hour in a sealed tube. The mixture is washed with water and dissolved in hot aqueous sodium hydroxide. The filtered solution is acidified with hydrochloric acid and the product is collected; yield 4.15 g. (98%). Recrystallization from boiling water (charcoal) gives an 85% recovery of spectroscopically pure product.

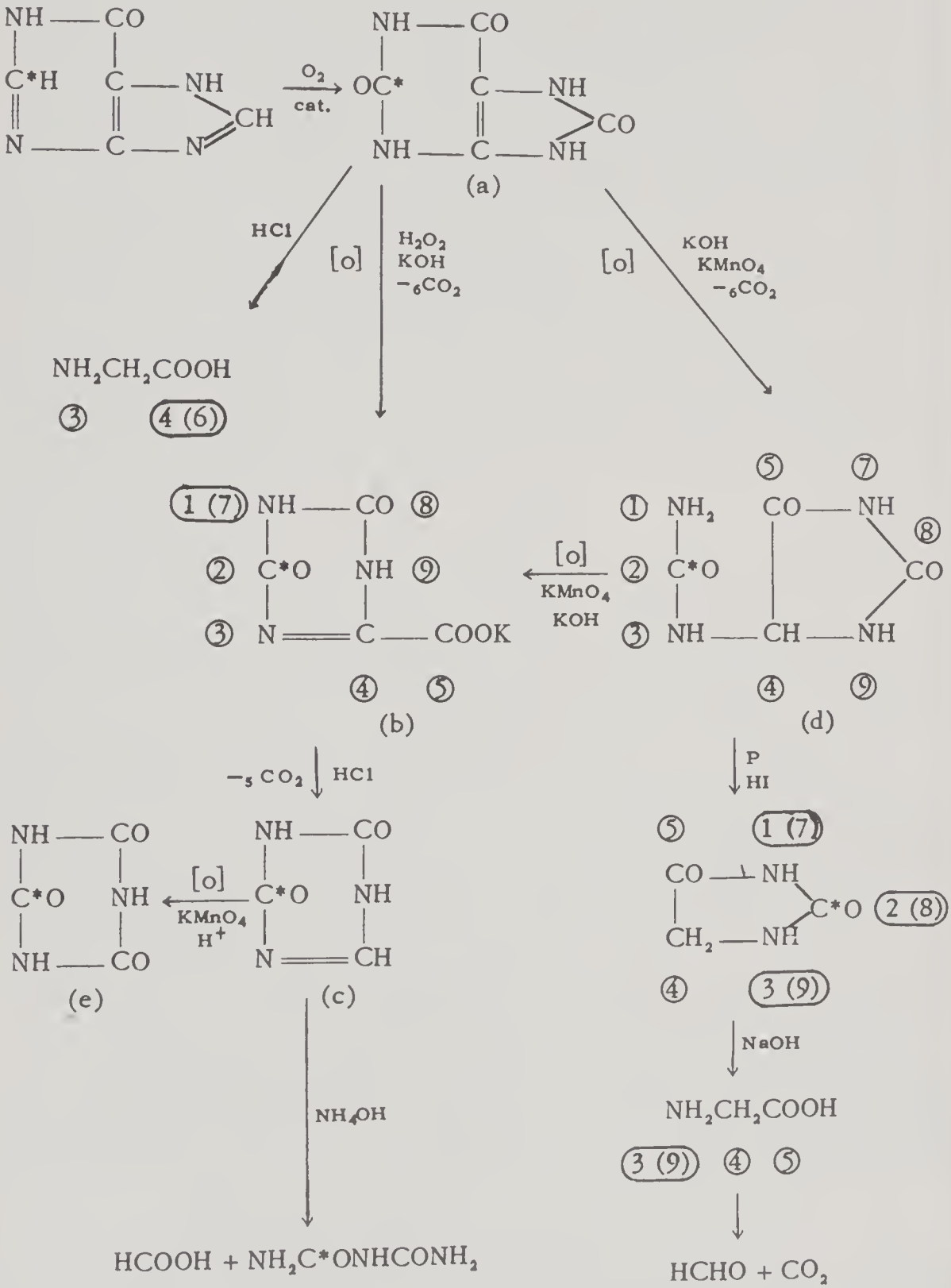
By the same method from ethyl cyanoacetate-2-C^{14} are prepared:[4,8] 6-aminouracil-5-C^{14}, 6-amino-5-nitrosouracil-5-C^{14}, 5,6-diaminouracil-5-C^{14} (yield 76%)[8] and uric-5-C^{14} acid; over-all yield[8] 13%. The molar extinction coefficients of C^{14}-uric acids are given.[8]

A preparation of uric-2-C^{14} acid has been reported by Canellakis.[8] A stirred mixture of 0.200 g. of urea-C^{14}, 0.323 g. of potassium methoxide and 0.6 ml. of absolute methanol is refluxed for 4 hours with a solution of 0.730 g. of ethyl acetamidomalonate in 1.3 ml. of absolute methanol. The 7-acetyluramil-2-C^{14} is hydrolyzed, and the uramil-2-C^{14}, (5-amino-barbituric-2-C^{14} acid), is condensed with potassium cyanate, forming 5-ureidobarbituric-2-C^{14} acid, which is isolated and converted to uric-2-C^{14} acid by the method of Fischer;[9] yield of product recrystallized from water, 51%. A sample recovered from a column of Dowex-1 (formate form) by gradient elution[10] with 1*M* ammonium formate exhibited only one radioactive peak. For other preparations of uric-2-C^{14} and -5-C^{14} acid, see allantoxaidine-2-C^{14}, Other Preparations.

The preparation of uric-8-C^{14} acid has been reported by Canellakis.[8] A finely ground mixture of 0.350 g. (2.44 mmoles) of uramil and 0.400 g. (6.67 mmoles) of urea-C^{14} is fused in the presence of 0.2 ml. of absolute methanol. The 5-ureido-C^{14}-barbituric acid is isolated and converted to uric-8-C^{14} acid by the method of Fischer;[9] yield of product recrystallized from water, 46% based on uramil. A sample recovered from a column of Dowex-1 (formate form) by gradient elution[10] with 1*M* ammonium formate exhibited only one radioactive peak.

[1]M. T. Bogert and D. Davidson, J. Am. Chem. Soc., *55*, 1667 (1933).
[2]T. B. Johnson and C. O. Johns, J. Am. Chem. Soc., *36*, 545 (1914).
[3]W. Traube, Ann., *432*, 266 (1923); Ber., *33*, 1371 (1900); M. Conrad, Ann., *340*, 310 (1905); H. Biltz and W. Schmidt, Ann., *431*, 94 (1923); B. Hepner and S. Frenkenberg, Helv. Chim. Acta, *15*, 350 (1932).
[4]C. E. Dalgliesh and A. Neuberger, J. Chem. Soc., *1954*, 3407.
[5]M. M. Stimson and M. A. Reuter, J. Am. Chem. Soc., *65*, 153 (1943).
[6]J. C. Sonne, J. M. Buchanan and A. M. Delluva, J. Biol. Chem., *173*, 69, 81 (1948); J. T. Sime and B. C. Johnson, J. Biol. Chem., *215*, 41 (1955); A. L. Krasna, P. Peyser and D. B. Sprinson, J. Biol. Chem., *198*, 421 (1952).
[7]H. Brandenberger, Biochim. et Biophys. Acta, *15*, 108 (1954).
[8]E. S. Canellakis and P. P. Cohen, J. Biol. Chem., *213*, 379, 387 (1955).
[9]E. Fischer, Ber., *30*, 559 (1897).
[10]R. B. Hurlbert, H. Schmitz, A. F. Brumm and V. R. Potter, J. Biol. Chem., *209*, 23 (1954).

ALLANTOXAIDINE-2-C^{14}



H. Brandenberger, Biochim. et Biophys. Acta, *15*, 108 (1954); *18*, 519 (1955); Helv. Chim. Acta, *37*, 641 (1954); H. Brandenberger and R. H. Brandenberger, Helv. Chim. Acta, *37*, 2207 (1954).

A. Procedure (Note 1)

(a) *Uric-2-C^{14} Acid*, [*2,6,8(1H, 3H, 9H)-purinetrione-2-C^{14}*]. Hypoxanthine-2-C^{14} (0.005 g.) is oxidized by shaking with an excess of xanthine oxidase (Note 2) in 300 ml. of 0.05 *M* glycylglycine buffer (pH 8) at room temperature in the presence of air, according to the method of Ball.[1] The reaction is followed by measuring the increase in optical density at 290 mμ (E 1.330 for the starting material). The product is isolated with the use of carrier and purified by repeated reprecipitation from aqueous piperidine solution with dilute hydrochloric acid (Note 3).

(b) *Potassium Allantoxanate-2-C^{14}, (Potassium Oxonate-2-C^{14})* (Note 4). To a solution of 0.017 g. (0.1 mmole) of uric-2-C^{14} acid (0.1 mmole) in 0.5 ml. of 2 *N* potassium hydroxide is added 0.25 ml. of 3% hydrogen peroxide. After standing for 2 days at room temperature in a stoppered tube, the mixture is decomposed with manganese dioxide, and the filtered solution is acidified with glacial acetic acid (Note 5) and stored in the cold. The product is collected and washed with ice water, alcohol and ether. Purification is effected by dissolution in a minimal volume of water at 50° and chilling with an ice-bath (Note 5); yield 0.008 g. (41%) (Note 6).

(c) *Allantoxaidine-2-C^{14}* (Note 7). To a concentrated aqueous solution of 0.360 g. of the potassium salt is added 3 ml. of 10% silver nitrate solution. The precipitated silver allantoxanate-2-C^{14} is collected by centrifuging and washed with water. To a suspension of the salt in 3 ml. of water is added 2 ml. of concentrated hydrochloric acid, and the mixture is warmed to 60° to hasten the decarboxylation. The supernatant solution is evaporated under vacuum, and the residue is washed with water, recrystallized from methanol and dried at 100° over phosphorus pentoxide; yield 0.109 g. (52%) (Note 8).

(d) *5-Ureido-C^{14}-hydantoin, (C^{14}_1-Allantoin)* (Note 9). To a stirred solution of 0.050 g. of uric-2-C^{14} acid in 2.5 ml. of 0.4 *N* potassium hydroxide is added 0.025 g. of potassium permanganate. The mixture is stirred for 10 minutes and filtered. The combined filtrate and wash liquor is acidified with 5 drops of glacial acetic acid (Note 4), heated briefly, concentrated to 1 ml. under vacuum and stored at 2° for 24 hours; yield 0.032 g. (Note 10).

B. Notes

1. These preparations are incidental to a tracer study of the mechanisms of alkaline oxidation of uric acid; also see Hartman,[2] Dalgliesh[3] and Canellakis.[13]

2. Directions are given by Ball[1] for preparing a high-activity concentrate of the enzyme from whole milk; 1.0 ml. is used.

3. Directions are given by Dalgliesh[3] for degrading the compound to glycine with strong acid. A degradation also is described by Buchanan.[4]

4. The procedure is adapted from the degradation of uric acid described by Venable[5] and Moore;[6] a similar isotopic procedure is described by Hartman. Apparatus for collection of isotopic ammonia and carbon dioxide is described by Brandenberger. As a result of degradative studies with the various labeled uric acids, forming the allantoxonic acids (b), allantoxaidines (c) and cyanuric acid (e), the classical imidazolidine structures[7] of (b) and (c) are replaced by the *s*-triazine formulas shown. The numbers in the formulae represent the source of the atoms derived from uric acid. An indirect method for calculating the complete N- and C-isotope distribution in labeled uric acid is given by Brandenberger.

5. The carbon dioxide evolved may be collected in saturated barium hydroxide solution under toluene by aeration with carbon dioxide-free air.

6. The formation of very small crystals, suitable for direct plating (assay), may be initiated by addition of several drops of alcohol.

7. These directions are Hartman's modification of those of Brandenberger, designed to permit easier separation of the inorganic acid and salt from the reaction products.

8. Directions are given by Hartman[2] for degrading allantoxaidine to formic acid (C-4) and biuret by alkaline hydrolysis; directions are also given for oxidizing it to cyanuric acid (e).

9. The method is an adaptation of that of Hartman, Moffett and Dickey.[8]

10. Allantoin prepared from uric-6-C^{14} acid is completely devoid of activity. Directions are given by Dalgliesh[3] for degrading the compound to hydantoin by reductive hydrolysis with phosphonium iodide.[9] Alkaline hydrolysis then gives glycine, which may be decarboxylated with ninhydrin, forming formaldehyde.

The degradation of allantoin to allantoxanic acid by alkaline permanganate oxidation is described by Brandenberger.

C. Other Preparations

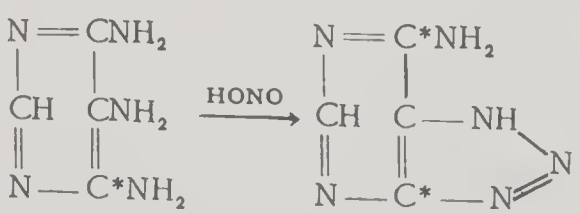
In a similar manner are prepared:[2,3,10] uric-4-C^{14} acid, uric-5-C^{14} acid, potassium allantoxanate-C^{14}, -4-C^{14}, -6-C^{14} (yield 63%), allantoxaidine-4-C^{14}, -6-C^{14}, and allantoin-2-C^{14}, -4-C^{14}, and -5-C^{14}. The requisite hypoxanthine-4-C^{14} was prepared[2] by the method of Shaw;[11] hypoxanthine-5-

C^{14} was prepared[2] by hydrolysis of the inosinic-5-C^{14} acid obtained from glycine-2-C^{14} by biosynthetic means.[1,2]

The preparation of 5-ureido-C^{14}-hydantoin, 5-ureidohydantoin-2-C^{14} and 5-ureidohydantoin-4-C^{14} from uric-2-C^{14}, -8-C^{14}, and -5-C^{14} acid by the described procedure has been reported by Canellakis.[13] The respective allantoins were reduced[13] with hydrogen iodide[14] to hydantoin-2-C^{14}, hydantoin-2-C^{14} and hydantoin-4-C^{14}; the molar specific activity of the hydantoin derived from uric-2-C^{14} or -8-C^{14} acid is one-half of that of the respective allantoin precursor. Oxidation of the respective allantoins gave potassium allantoxanate-2-C^{14}, -6-C^{14}, and allantoxanate-C^{14}. The carbon-C^{14} dioxide evolved by decarboxylation of potassium allantoxanate-C^{14}, derived from uric-5-C^{14} acid, has the same molar specific activity as its three precursors.

[1]E. G. Ball, J. Biol. Chem., *128*, 51 (1939).
[2]S. C. Hartman and J. Fellig, J. Am. Chem. Soc., 77, 1051 (1955).
[3]C. E. Dalgliesh and A. Neuberger, J. Chem. Soc., *1954*, 3407.
[4]J. M. Buchanan, J. C. Sonne and A. M. Delluva, J. Biol. Chem., *173*, 81 (1948).
[5]C. S. Venable, J. Am. Chem. Soc., *40*, 1099 (1918).
[6]F. J. Moore and R. M. Thomas, J. Am. Chem. Soc., *40*, 1120 (1918).
[7]H. Biltz and H. Schauder, J. prakt. Chem., *106*, 108 (1923); H. Biltz and R. Robl, Ber., *53*, 1967 (1920).
[8]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 21.
[9]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 489.
[10]H. Brandenberger.
[11]E. Shaw and D. W. Woolley, J. Biol. Chem., *181*, 89 (1949); E. Shaw, J. Biol. Chem., *185*, 439 (1950).
[12]M. P. Schulman, J. C. Sonne and J. M. Buchanan, J. Biol. Chem., *196*, 499 (1952).
[13]E. S. Canellakis and P. P. Cohen, J. Biol. Chem., *213*, 379 (1955).
[14]L. F. Cavalieri, V. E. Blair and G. B. Brown, J. Am. Chem. Soc., *70*, 1240 (1948).

7-AMINO-1*H*-*v*-TRIAZOLO[*d*]PYRIMIDINE-3a, 7-$C^{14}_{1/2}$
(8-Azaadenine-4,6-$C^{14}_{1/2}$)



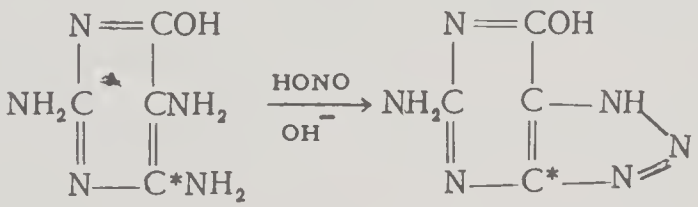
E. L. Bennett, J. Am. Chem. Soc., *74*, 2420 (1952); Nucleonics, 7 (4), 49 (1950).

Procedure

To a solution of 0.608 g. of 4,5,6-triaminopyrimidine-4-C^{14} sulfate in 10 ml. of 2 *M* sodium acetate at room temperature is added 1.5 ml. of glacial acetic acid and 0.213 g. of sodium nitrite in 5 ml. of water. The mixture is heated for 20 minutes on a steam-bath and set aside at 5° to crystallize. The product is filtered off, washed with a small amount of cold water and alcohol, and dried; yield 0.295 g. (86%). The product is decolorized with 0.05 g. of Norit in warm *N* ammonium hydroxide solution; then glacial acetic acid is added to the warm solution until precipitation begins. The pure product, collected from the cooled mixture in 0.270 g. yield (79%), decomposes[1] above 310° without melting. Reworking the mother liquor with 0.051 g. of carrier yields 0.052 g. of product of lower (23%) specific activity, making the total radiochemical yield 82%. Paper chromatography shows the presence of only one radioactive compound.

[1]J. O. Roblin, Jr., J. O. Lampen, J. P. English, Q. P. Cole and J. R. Vaughan, Jr., J. Am. Chem. Soc., 67, 290 (1945).

5-AMINO-1*H*-*v*-TRIAZOLO[*d*]PYRIMIDIN-7-ol-3a-C^{14} (8-Azaguanine-4-C^{14})



E. L. Bennett, J. Am. Chem. Soc., 74, 2420 (1952); Nucleonics, 7 (4), 49 (1950).

A. Procedure

To a solution of 1.36 g. of 2,5,6-triamino-4-pyrimidinol-6-C^{14} sulfate, in 30 ml. of 2 *M* sodium acetate at 80°, is added a solution of 1.4 g. of sodium nitrite in 8 ml. of water, and the mixture is heated for 30 minutes on a steam-bath. Glacial acetic acid (4 ml.) is added, and the product is crystallized at 5°. The solid is collected, washed with cold water, dissolved in warm *N* ammonium hydroxide, decolorized with charcoal and again crystallized at 5°; yield 0.556 g. (69%). An additional 0.194 g. is obtained by acidifying the warmed mother liquor with glacial acetic acid to the point of precipitation, making the total yield 93%. Filter paper chromatography shows the presence of only one radioactive compound.

B. Other Preparations

In a similar manner is prepared[1] 8-azaguanine-2-C^{14} from 2,5,6-triamino-4-pyrimidinol-2-C^{14}; yield 67%.

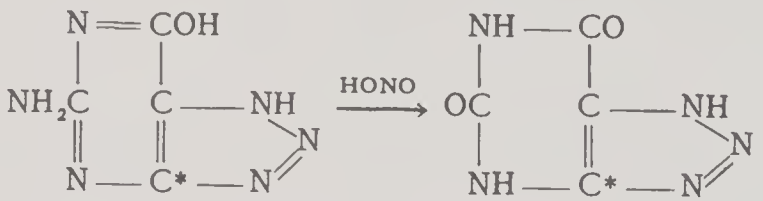
Mandel[2] has prepared 8-azaguanine-4-C^{14} (89% yield) by essentially this procedure,[3] but purified the product by repeated extraction of the reaction mixture with 1-butanol at various pH values. The product was shown to be pure by chromatography, 8-plate countercurrent distribution and ultraviolet absorption data.

[1]L. L. Bennett, Jr., H. E. Skipper, J. H. Mitchell, Jr. and K. Sugiura, Cancer Research, *10*, 644 (1950); Nucleonics 7 (4), 49 (1950).

[2]H. G. Mandel, E. L. Alpen, W. D. Winters and P. K. Smith, J. Biol. Chem., *193*, 63 (1951).

[3]R. O. Roblin, Jr., J. O. Lampen, J. P. English, Q. P. Cole and J. R. Vaughan, Jr., J. Am. Chem. Soc., 67, 290 (1945).

1*H*-*v*-TRIAZOLO[*d*]PYRIMIDINE-5,7(4*H*,6*H*)-DIONE-3a-C^{14} (8-Azaxanthine-4-C^{14})



H. G. Mandel, E. L. Alpen, W. D. Winters and P. K. Smith, J. Biol. Chem., *193*, 63 (1951).

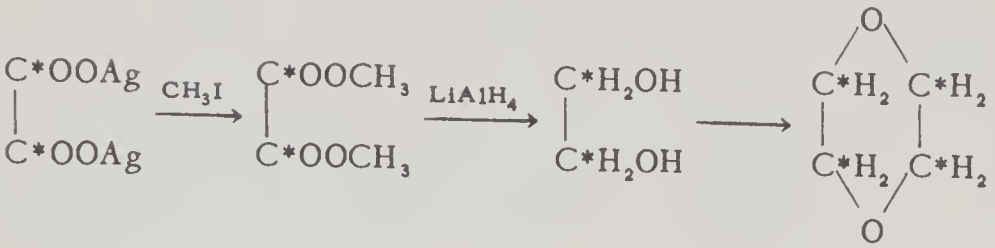
Procedure

1*H*-*v*-Triazolo[*d*]pyrimidine-5,7(4*H*,6*H*)-dione-3a-C^{14} [and 5-C^{14}] are prepared from the respective azaguanines, 5-amino-1*H*-*v*-triazolopyrimidin-7-ol-3a-C^{14} [and 5-C^{14}], by the procedure of Roblin,[1] which follows. Both the ultraviolet absorption spectrum and paper chromatography-radioautography indicate that the product is homogeneous.

To a solution of 0.55 g. (3.6 mmoles) of 5-amino-1*H*-*v*-triazolo[*d*]-pyrimidin-7-ol in 10 ml. of 15% sulfuric acid, prepared by boiling and cooling to 60–70°, is added a solution of 0.5 g. (7.2 mmoles) of sodium nitrite in 2 ml. of water in small portions with vigorous shaking. When there is no further gas evolution, the solution is cooled for 2 hours to crystallize the crude product; yield 0.46 g. (74%). Recrystallization from 15 ml. of water gives 0.29 g. (47%) of the colorless monohydrate, which decomposes above 270° without melting.

[1]R. O. Roblin, Jr., J. O. Lampen, J. P. English, Q. P. Cole and J. R. Vaughan, Jr., J. Am. Chem. Soc., 67, 290 (1945).

p-DIOXANE-C_4^{14}



(a) (b) (c)

W. Stumpf, F. Weygand and O. A. Grosskinsky, Ber., 86, 1391 (1953).

A. Procedure

(a) *Methyl Oxalate-C_2^{14}.* The ester is obtained in approximately 100% radiochemical yield by treating silver oxalate-C_2^{14} with an excess of methyl iodide, and isolating with the aid of carrier. For a similar reaction see ethyl malonate-2-C^{14}.

(b) *1,2-Ethanediol-C_2^{14}, (Ethylene-C_2^{14} Glycol).* To a stirred, turbid solution of 0.320 g. of lithium aluminum hydride in 10 ml. of absolute ether is added dropwise 0.162 g. of methyl oxalate-C_2^{14} in 5 ml. of absolute ether. The mixture is refluxed for 45 minutes, treated with 2 ml. of 1.9 *M* lithium aluminum hydride solution at 30 minutes and stored overnight. The mixture is cooled with an ice-bath and treated with a mixture of 10 ml. of redistilled methanol, 0.775 ml. of water and 2 ml. of distilled glycol; then carbon dioxide is bubbled briskly through the mixture for 1.5 hours, removing most of the ether through the condenser. The mixture is transferred into the thimble of a 30-ml. Soxhlet extractor and extracted for 8 hours with methanol. The filtered extract (pH 7.0) is fractionally distilled on a water-bath through a 25-cm. Widmer spiral-fractionating column to remove the methanol. The residue (7 ml.) is fractionally distilled with an oil-bath temperature up to 160°; then the fractionating column is replaced by a simple, short distilling head, and the residue (4 ml.) is distilled at 13 mm. into an ice-cooled receiver. The upper part of the distilling flask is brushed with a flame; then 0.5 ml. of carrier glycol is added and distilled out, leaving about 50 ml. of brown residue. The crude product is purified by redistillation. When the vapor temperature reaches 194° in the neck of the flask (bath 210°), any water condensate is removed by brushing with a flame, the bath is removed, and the upper neck of the flask is washed out with methanol and sucked dry. The product then is collected in a receiver protected with a calcium chloride tube, the system being chased with 0.5 ml. of carrier glycol; yield 2.78 g., b.p. 195° (cor.), radiochemical yield 30.2%.

The product is assayed as ethylene-C_2^{14} benzoate, m.p. 71.5°, prepared by adding 0.12 ml. of benzoyl chloride dropwise to a stirred solution of 0.014 ml. of the glycol in 1 ml. of pyridine, treating with sodium bicarbonate at 30 minutes, then collecting and washing with water after 3 hours.

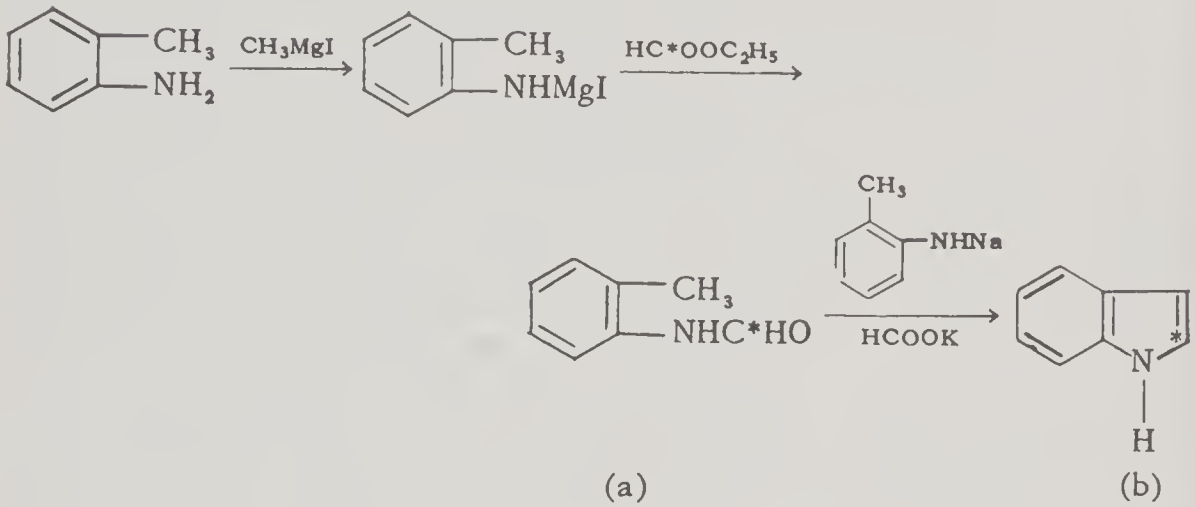
(c) p-*Dioxane*-C_4^{14}. The apparatus (see reference) consists of a distilling flask equipped with a pressure-equalizing addition funnel and nitrogen inlet, a water-cooled condenser and a receiver, attached in series to a gas-washing bottle, a trap containing *p*-nitrophenylhydrazine and acetic acid, a barium hydroxide trap, and a guard tube containing soda lime and calcium chloride. A mixture of 5 ml. of ethylene-C_2^{14} glycol and 0.1 ml. of concentrated sulfuric acid is heated in an oil-bath from 190° to 220°, and the remainder of the total of 26.46 g. of the glycol is added dropwise at the same rate as the distillation of the crude product, which requires 1.5 hours. The dry, black residue is removed; the crude dioxane is transferred back into the distilling flask and redistilled at a rising bath temperature of 110° to 170°. To the residue (essentially one-half of the initial glycol) is added 4 drops of sulfuric acid, and the product is distilled slowly with a rising bath temperature to 220°. The process is repeated five times more with additions of one drop of sulfuric acid, until no further glycol is fractionated by distillation. The colorless crude product is shaken with 5 g. of potassium hydroxide pellets in a small separatory funnel, until two layers form and brown flakes precipitate. After one day, the brown lower layer is separated and the upper layer is filtered through sintered glass. The pale-yellow solution is refluxed for 1 day under nitrogen with 5 g. of potassium hydroxide to resinify the contaminant (unnecessary in trial runs). The upper layer is separated and distilled, yielding 18.0 g. of crude *p*-dioxane-C_4^{14}, b.p. 100°.

Purification is accomplished by refluxing for 10 hours with 1.8 ml. of *N* hydrochloric acid in a gentle stream of nitrogen, which is introduced about 5 mm. above the liquid level. The acetaldehyde formed is swept through the condenser and absorbed in the exhaust purifying train described above. The cooled mixture is treated with potassium hydroxide pellets for 1 day; then the still-colored upper layer is separated and distilled from potassium hydroxide. The colorless distillate once more is refluxed over potassium hydroxide for 10 hours. The distilled product is stored over 1 g. of sodium wire for 1 day, then refluxed over sodium under nitrogen for 1.5 days. The distilled product is next refluxed for 0.5 day with fresh sodium, and again distilled; yield 10.4 g. (55.4%), b.p. 100°; in model experiments the yield was 79%. The ultraviolet absorption spectrum shows that the purity is less than that of technical

dioxane, which has been carefully purified on a larger scale and fractionally distilled.

INDOLE-2-C^{14}

METHOD I



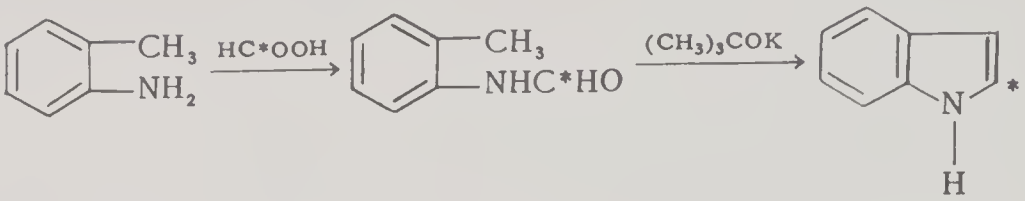
L. Pichat, M. Audinot and J. Monnet, Bull. soc. chim. France, (5) *21*, 85 (1954).

A. Procedure (Note 1)

(a) *o-Formotoluidide-1-*C^{14} (Note 2). To the stirred, ice-cooled solution of o-toluidinemagnesium iodide, prepared from 0.415 g. of magnesium, 2.4 g. of methyl iodide, 60 ml. of absolute ether and 1.79 g. of o-toluidine, is added 9.8 mmoles of ethyl formate-C^{14} by distillation in a gentle stream of dry nitrogen (Note 3). The mixture is stirred at room temperature for 30 minutes and decomposed with a concentrated aqueous solution of ammonium chloride; yield 1.40 g. (99.2%).

(b) *Indole-2-*C^{14} (Note 4). A mixture of 9 g. of anhydrous *o*-toluidine and 1.17 g. of sodium hydride is heated at 160° in a slow stream of dry nitrogen until no further hydrogen is evolved (30 minutes). To the sodium *o*-toluidide is added the crude o-formotoluidide-1-C^{14} and 3.6 g. of freshly fused potassium formate (Note 5). The mixture is heated under reflux to 300–305° during 30 minutes (Note 6), and the excess o-toluidine is then distilled into a receiver. The cooled mixture is diluted with water, and the product is steam-distilled The distillate is extracted with ether, and the extract is washed with *N* hydrochloric acid to remove traces of *o*-toluidine, and evaporated under reduced pressure; yield 80% (Note 7). The molar specific activity is the same as that of the starting material.

METHOD II



(a) (b)

E. Leete and L. Marion, Can. J. Chem., *31*, 1195 (1953).

A. Procedure (Note 8)

(a) *o-Formotoluidide-1-C*^{14}. A mixture of 0.010 g. of *o*-toluidine hydrochloride, 2.40 g. (2.22 mmoles) of freshly distilled *o*-toluidine and 0.00492 g. of sodium formate-C^{14} is heated for 1 minute on a steam-bath. To the mixture is added 0.92 g. (2.00 mmoles) of 99% formic acid, and heating is continued for 12 hours in an atmosphere of dry nitrogen. The mixture is cooled and treated with 1% hydrochloric acid to crystallize the product which is collected, washed with water and dried under vacuum; yield 2.2225 g., 82.5%.

(b) *Indole-2-C*^{14}. The *o*-formotoluidide-1-C^{14} is added to a solution of 1.1 g. of potassium in 20 ml. of *t*-butyl alcohol under nitrogen. The excess solvent is distilled off, and the residue is heated slowly to 350–360° until effervescence ceases (15 minutes). The mixture is cooled, water is added, and the product is collected by steam distillation; yield 0.6081 g. (31.4%). The molar specific activity appears to be 10.3% of that of the starting material. Degradation studies indicate that there is no isotopic rearrangement (Note 9).

B. Notes

1. The method is a modification of that of Heidelberger; see indole-3-C^{14}.

2. The procedure is an adaptation of that of Bodroux[1] for preparing amides.

3. The apparatus consists of a round-bottomed flask fitted with a Dry Ice reflux condenser, an addition tube, a magnetic stirrer, a drying tube, and a gas inlet delivering at the bottom and connected from the top of another reflux condenser; a diagram is given by Pichat. The C_1^{14} ester is entrained in a nitrogen stream as formed, by heating a mixture of 9.8 mmoles of dry sodium formate-C^{14} and 10 ml. of ethyl sulfate at 155–160° under reflux for 3 hours.

4. The method is a modification of that of Tyson.[2] A diagram of the apparatus is given by Pichat.

5. In a trial preparation using potassium formate-C^{14} and nonisotopic o-formotoluidide, only 2% of the activity entered the indole, which was formed in 83% yield. The role of the salt in the reaction appears to be catalytic.[3]

6. The evolved hydrogen and carbon monoxide are inactive.

7. The residual tar contains 15% of the initial activity.

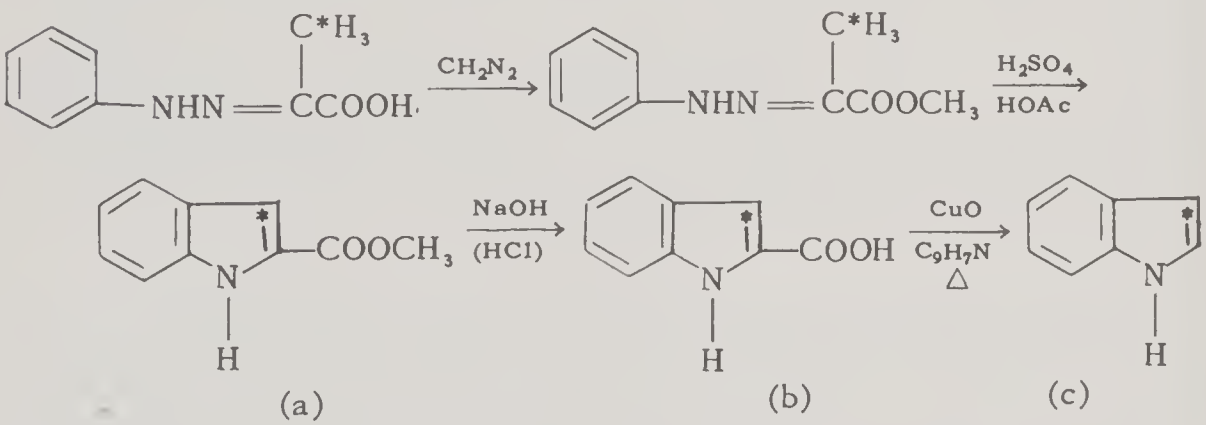
8. See the similar preparation of *o*-formotoluidide-α-C^{14} and indole-3-C^{14}.

9. Directions are given by Leete for converting[4] the indole-2-C^{14} to 1-benzoylindole-2-C^{14}, having the same molar specific activity, by treatment of the sodium indole-2-C^{14} with benzoyl chloride. Oxidation[5] of this compound with potassium permanganate in acetone solution yields inactive *N*-benzoylanthranilic acid.

[1]F. Bodroux, Compt. rend., *138*, 1427 (1904).
[2]F. T. Tyson, J. Am. Chem. Soc., *72*, 2801 (1950).
[3]A. Galat and H. L. Friedman, J. Am. Chem. Soc., *70*, 1280 (1948).
[4]R. Weissgerber, Ber., *43*, 3520 (1910).
[5]R. Weissgerber, Ber., *46*, 651 (1913).

INDOLE-3-C^{14}

METHOD I



E. Leete, L. Marion and I. D. Spenser, Can. J. Chem., *33*, 405 (1955).

A. Procedure

(a) *Methyl 2-Indolecarboxylate-3-*C^{14} (Note 1). Pyruvic-3-C^{14} acid phenylhydrazone (1.382 g.) is esterified in ether suspension with excess diazomethane, and the solvent is removed by distillation.

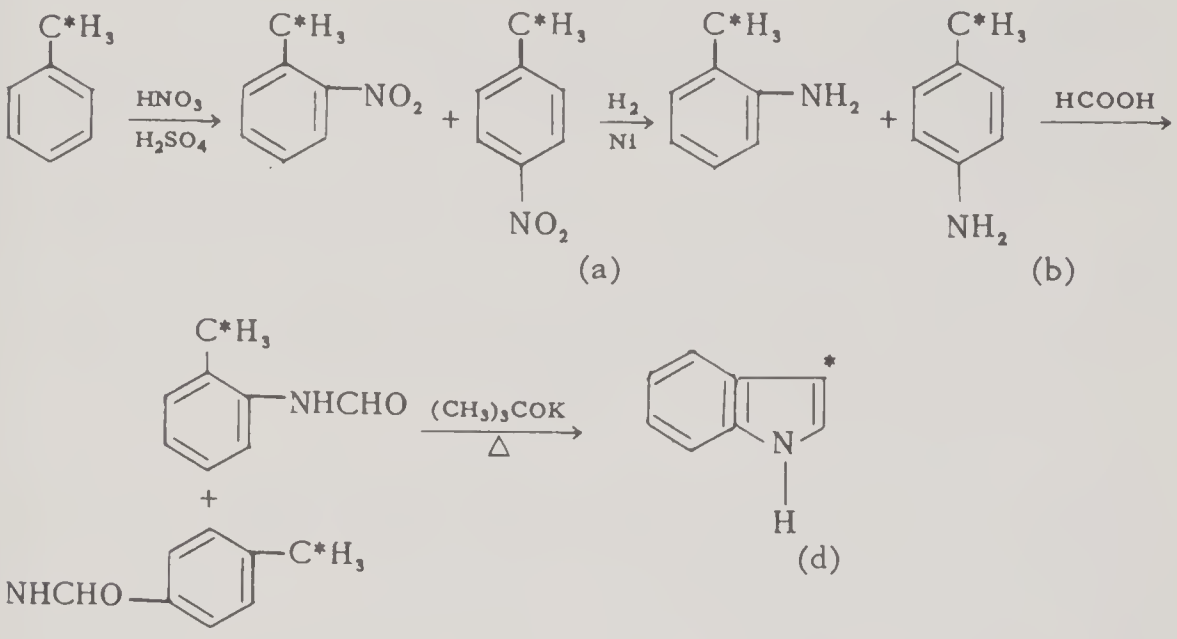
To a cooled solution of the residual crude methyl pyruvate-3-C^{14} phenylhydrazone in 6 ml. of glacial acetic acid is added 0.7 ml. of concentrated sulfuric acid. The solution is heated at 80° for 1 hour (Note 2), filtered, cooled, diluted with 250 ml. of water and 50 ml. of 2 *N* ammonium hydroxide, and stored at 0° for 48 hours. The product is col-

lected and sublimed at 120–140° (1 μ), the sublimate being collected with methanol and evaporated.

(b) *2-Indolecarboxylic-3-C^{14} Acid.* The ester is hydrolyzed with 10 ml. of hot *N* sodium hydroxide; then the mixture is acidified with 12 ml. of 2 *N* hydrochloric acid and stored in a refrigerator for 36 hours. The crude product is collected and dried over concentrated sulfuric acid; yield 0.371 g. (29.7% based on the phenylhydrazone), m.p. 176–179°.

(c) *Indole-3-C^{14}* (Note 3). A mixture of the acid, 0.020 g. of cupric oxide and 6 ml. of quinoline is refluxed under nitrogen for 16 hours, then cooled and diluted with 200 ml. of ether. The filtered solution is washed with sodium hydroxide and dilute hydrochloric acid, then dried over sodium sulfate and evaporated under vacuum; yield 0.290 g.

METHOD II



C. Heidelberger, J. Biol. Chem., *179*, 139 (1949).

A. Procedure

(a) o- *and* p-*Nitrotoluene-α-C^{14}.* Toluene-α-C^{14} is nitrated in 58% yield by the procedure of Hickinbottom,[1] which follows.

To 20 g. of well stirred toluene, maintained at 30°, is added slowly a cold mixture of 20 g. of nitric acid (d. 1.42) and 30 g. of concentrated sulfuric acid. The temperature is gradually raised and maintained at 50° for 1 hour. The mixture is cooled, and the product layer is separated and washed with water, dilute sodium carbonate solution and water. The

product is a mixture of about 63% ortho-, 35% para-, and 2% meta-compounds (Note 4).

(b) o- *and* p-*Toluidine-α-C*^{14}. The mixture of *o*- and *p*-nitrotoluene-α-C^{14} is hydrogenated in benzene at 210° over a nickel-kieselguhr catalyst as described by Covert.[2]

(c) o- *and* p-*Formotoluidide-α-C*^{14}. The mixture of crude *o*- and *p*-toluidine-α-C^{14} is formylated by heating on a steam-bath for 3 hours with a slight excess of 90% formic acid, according to the procedure of Tyson[3] (Note 5). The isotopic yield is 65%.

(d) *Indole-3-C*^{14}. The intramolecular condensation of *o*-formotoluidide-α-C^{14} is effected in the presence of potassium *t*-butoxide by the procedure of Tyson[3] (Note 6). Only the ortho-isomer of the formotoluidide mixture reacts. The isotopic yield is 0.39 g., 33% (Note 7).

B. Notes

1. The method is an adaptation of that of Elks.[4]

2. The crystallization of ammonium acetate is complete in this time.

3. A survey of a number of methods for preparing indole is presented by Van Order.[5]

4. Vacuum distillation leaves a residue enriched with the para-form, which crystallizes upon cooling to 0°. The distillate is enriched with the ortho-isomer, which crystallizes out upon cooling to −15°. The rate of nitration under various conditions has been determined by Ingold.[6]

5. The yield is raised from 85–89% to 100% by using 99% formic acid; also see indole-2-C^{14}.

6. The yield is 79%, based on the assumption that two moles of *o*-formotoluidide are required by theory to form one mole of indole. The yield is decreased by using other potassium alkoxides, while the amide, monoxide, and alkoxides of sodium are unsuccessful as condensation reagents. Recent studies, have thrown more light upon the effect of condensing agents upon this reaction, and yields have been raised considerably; also see indole-2-C^{14}, Method I.

7. A degradation of indole is described by Yanofsky.[8]

[1]W. J. Hickinbottom, *Reactions of Organic Compounds*, Longmans, Green and Co., London, 1936, p. 37.

[2]L. W. Covert, R. Connor and H. Adkins, J. Am. Chem. Soc., *54*, 1651 (1932).

[3]*Organic Syntheses*, Vol. 23, Wiley, New York, 1943, p. 42.

[4]J. Elks, D. F. Elliott and B. A. Hems, J. Chem. Soc., *1944*, 629.

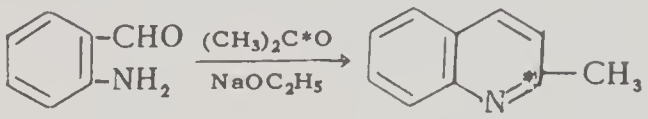
[5]R. B. Van Order and H. G. Lindwall, Chem. Revs., *30*, 69 (1942).

[6]C. K. Ingold, A. Lapworth, E. Rothstein and D. Ward, J. Chem. Soc., *1931*, 1959.

[7]F. T. Tyson, J. Am. Chem. Soc., *72*, 2801 (1950); A. Galat and H. L. Friedman, J. Am. Chem. Soc., *70*, 1280 (1948).

[8]C. Yanofsky, J. Biol. Chem., *217*, 345 (1955).

QUINALDINE-2-C^{14}
(2-Methylquinoline-2-C^{14})



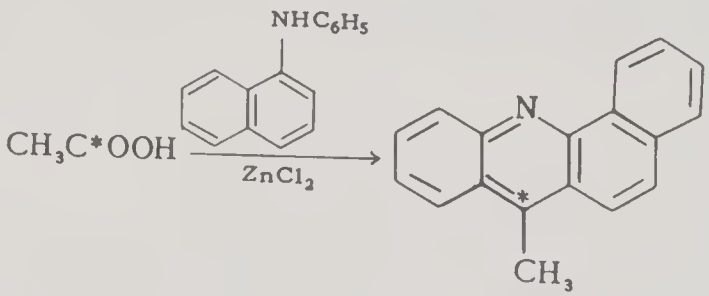
W. G. Dauben and C. W. Vaughan, Jr., J. Am. Chem. Soc., 75, 4651 (1953).

Procedure

An ampoule containing the 2-propanone-2-C^{14} prepared from 12.3 mmoles of acetic-1-C^{14} acid is broken beneath the surface of a stirred solution of 3.098 g. (24.8 mmoles) of *o*-aminobenzaldehyde in 400 ml. of 95% alcohol. The mixture is treated with a solution of 5 g. (220 mmoles) of sodium in 100 ml. of 95% alcohol and stirred for 48 hours. The mixture is acidified with concentrated hydrochloric acid, and the solvent is distilled through a Vigreux column. The residue is made basic with 50 ml. of 1 *N* sodium hydroxide, and the product is extracted into ether solution and dried over magnesium sulfate. The extract is diluted with 80 g. of carrier, and the mixture is fractionally distilled; yield 74.5 g., b.p. 119–121° (16 mm.).

[1]*Organic Syntheses*, Vol. 28, Wiley, New York, 1948, p. 11.

7-METHYLBENZ[*c*]ACRIDINE-7-C^{14}



P. Daudel, A. Cheutin, M. Flon, N. P. Buu-Hoi, and R. Muxart, Bull. soc. chim. France, *19*, 86 (1952).

A. Procedure

A mixture of 0.025 g. of sodium acetate-1-C^{14}, 0.04 ml. of concentrated hydrochloric acid, 0.120 g. of fused zinc chloride and 0.040 g. of *N*-phenyl-1-naphthylamine is heated in a sealed tube for 15 hours at 180° (Note 1). The tube is cooled and evacuated at a water pump so that any vapors are trapped in an alkali bubbler. The reaction mixture is made basic with about 0.200 g. of soda in 2 ml. of water, 1 ml. of toluene is added and the mixture is warmed with stirring (glass rod). The liquid phases are transferred to a centrifuge tube with toluene, and the

organic layer is separated with the use of centrifuge and pipette. The solvent is removed on a water-bath at reduced pressure (Note 2)[7] and the residue is refluxed for 15 hours with petroleum ether, which has been previously purified by passage through a column of alumina. The extract is separated by chromatography on a column of alumina (Note 3). The product affixes itself at the top of the column and exhibits yellowish fluorescence, while the excess amine affixes itself beneath this band and fluoresces blue. The tube is cut at the interface of the two zones, and the product is extracted by repeated and prolonged refluxing with successive portions of purified petroleum ether (Note 4). The product is crystallized by evaporation of solvent, the yield being of the order of a few milligrams. The ultraviolet absorption spectrum indicates less than 1% contamination with the starting amine.

B. Notes

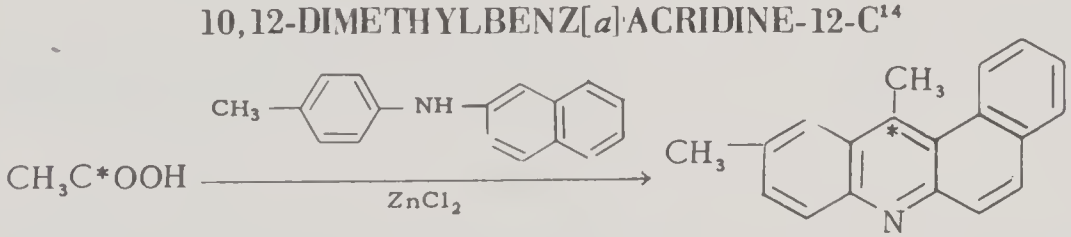
1. This procedure is the same as that employed in the preparation of 10,12-dimethylbenz[*a*]acridine-12-C^{14}. The sodium acetate is introduced to the reaction tube in a weighed capillary tube, and the mixture of zinc chloride and amine is introduced in a similar manner. The whole is then mixed with a heavy glass rod, and the acid is added with a capillary pipette. A diagram of the reaction tube, sealed at one end with a standard taper plug and equipped with a long side arm with a stopcock at its outer end, is presented by Daudel. The tube is heated in a furnace.

2. A liquid-nitrogen trap is employed to prevent possible contamination of the water pump with radioactive material.

3. The simple column has a diameter of 3 mm. and is fitted with a funnel.

4. The petroleum ether is purified by passage through a column of alumina, followed by digestion with fresh alumina.

10,12-DIMETHYLBENZ[*a*]ACRIDINE-12-C^{14}



P. Daudel, M. Flon, A. Cheutin, R. Muxart, N. P. Buu-Hoi, B. Eckert and N. Hoan, Compt. rend., *232*, 968 (1951).

A. Procedure

A mixture of 0.025 g. of sodium acetate-1-C^{14}, 0.04 ml. of concentrated hydrochloric acid, 0.120 g. of fused zinc chloride and 0.040 g. of *N*-*p*-tolyl-2-naphthylamine is heated in a sealed Pyrex tube at 190° for 20

hours (Note 1). The mixture is made basic with sodium carbonate solution (about 0.200 g. in 2 ml. of water) in the presence of 1 ml. of toluene. The organic layer is separated and evaporated on a water-bath under reduced pressure. The residue is refluxed for 15 hours with petroleum ether; then the solution is fractionated by chromatography on a column of alumina prepared with petroleum ether. The column is cut between the two fluorescent bands (Note 2), and the product is eluted with boiling petroleum ether and with benzene. Evaporation of the solvents yields a few milligrams of product from each extract (Note 3). Spectroscopic examination shows that the product crystallized from the petroleum ether has a purity of 99%, which is higher than that from the benzene.

B. Notes

1. The procedure is a modification[1,2] of the Bernthsen[3] method of preparing acridines (see 7-methylbenz[*c*]acridine-7-C^{14}). Use of the isomeric *N*-*p*-tolyl-1-naphthylamine forms 7,9-dimethylbenz[*c*]acridine.

2. The product, with yellowish fluorescence, appears above a second band of blue fluorescence.

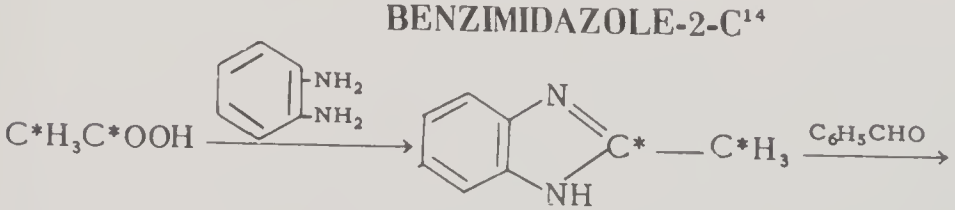
3. Silky, almost colorless needles,[1] m.p. 140°; picrate, m.p. 226–227°.

[1]N. P. Buu-Hoi and J. Lecoeq, Compt. rend., *218*, 792 (1944); N. P. Buu-Hoi, J. Chem. Soc., *1946*, 792; *1949*, 670.

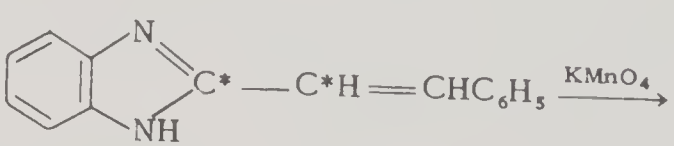
[2]Daudel, Flon, Cheutin, Muxart, Buu-Hoi, Melet and Eckert, Congr. intern. biochim., Resumés communs, 2^{e} Congr., Paris, *1952*, 466.

[3]A. Bernthsen, Ann., *224*, 1 (1884).

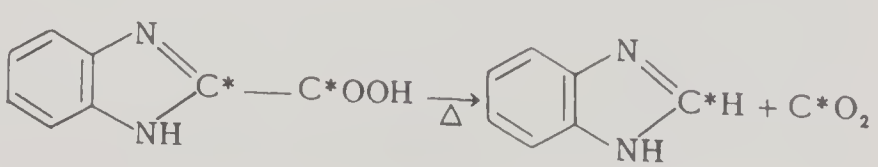
BENZIMIDAZOLE-2-C^{14}



(a)



(b)



(c) (d)

S. Roseman, J. Am. Chem. Soc., 75, 3854 (1953).

A. Procedure (Note 1)

(a) *2-Methyl-$C^{14}_1/_1$-benzimidazole-2-$C^{14}_1/_1$.* A mixture of 0.400 g. of acetic-$C^{14}_1/_2$ acid, 4.0 g. of *o*-phenylenediamine dihydrochloride (Note 2), 10 ml. of water and 1.5 ml. of phosphoric acid (85%) is heated in a sealed tube for 2 hours at 135°. The tube is cooled, opened and heated in an oil-bath for 2 hours at 135°. The thick syrup is dissolved in water and neutralized with solid potassium carbonate, and the pH is adjusted to 3.5–4.0 with glacial acetic acid. The solution is shaken with 10 ml. of benzaldehyde for 30 seconds and extracted with three 70-ml. portions of chloroform. The benzaldehyde treatment is repeated (2–3 times) until no further color develops. The filtered, colorless, aqueous solution is washed with petroleum ether and neutralized with solid potassium carbonate. Addition of 30 ml. of concentrated ammonium hydroxide, and a solution containing 4 g. of silver nitrate, 4 g. of water and 6 g. of ammonium hydroxide precipitates a colorless silver salt (Note 3), which is quickly centrifuged and thoroughly washed with water. A suspension of the salt in alcohol-water is saturated with hydrogen sulfide. The solution is decolorized with a very small amount of charcoal and evaporated to dryness with the addition of 1 ml. of ammonium hydroxide toward the end. The yield of colorless needles is 0.750 g., m.p. 174–176° (cor.). The product is purified by recrystallization from dilute ammonium hydroxide solution, or by vacuum-sublimation at 130–145° (10 μ), m.p. 175.5–176.5° (Note 4).

(b) *2-(Styryl-β-$C^{14}_1/_1$)benzimidazole-2-$C^{14}_1/_1$.* 2-Methyl-$C^{14}_1/_1$-benzimidazole-2-$C^{14}_1/_1$ is heated for 2 hours at 190–200° with three times its weight of benzaldehyde in a sealed tube. The oil is dissolved in a 1:1 acetone-ether mixture, and an excess of concentrated sulfuric acid is added with vigorous mixing. The precipitate is centrifuged, washed with acetone-ether and dried. The salt is heated with four volumes of 0.4 *N* sulfuric acid on a steam-bath, crystallized by storing for several hours at 0°, centrifuged, and washed with ice-water. The salt is dissolved in a minimum amount of hot alcohol containing an excess of concentrated ammonium hydroxide; the addition of 3–4 volumes of hot water precipitates a colorless oil which crystallizes in the refrigerator, m.p. 200–201°. The yield is 0.200 g. and 0.011 g. from 0.295 g. and 0.013 g., respectively, of 2-methylbenzimidazole.

(c) *2-Benzimidazolecarboxylic-$C^{14}_1/_1$-2-$C^{14}_1/_1$ Acid.* To a cold mixture of 0.060 g. of the olefin, 5 ml. of pyridine, and 0.010 g. of potassium carbonate is added 5 ml. of potassium permanganate solution (10% excess), and the mixture is kept at 0° for 2 hours. The pyridine is removed by steam-distillation, the excess permanganate is destroyed with a few drops of alcohol, and the mixture is filtered at a boil with the aid of

Celite. The filtrate is adjusted to pH 6 with acetic acid and stored at 4° for 2 days. The product crystallizes as long colorless needles, which are dried at room temperature (Note 5); yield about one-half the weight of the starting material, m.p. 174° (dec.).

(d) *Benzimidazole-2-C*^{14}. 2-Benzimidazolecarboxylic-C^{14}_1-2-C^{14}_1 acid is decarboxylated by heating at the melting point. The evolved carbon-C^{14} dioxide originates as C-2 of acetic acid, while the benzimidazole contains C-1.

B. Notes

1. The original paper describes the preparation of singly labeled methylbenzimidazole from acetic-1-C^{14} acid, and from acetic-2-C^{14} acid, in the development of this method for degrading and characterizing micro-amounts of impure labeled acetic acid. Acetic-C^{14}_2 acid is used for convenience in illustrating the method.

2. The molar ratio of *o*-phenylenediamine to acetic acid can be varied from 2:1 to 6:1.

3. The salt darkens quickly if the purification by the benzaldehyde treatment is incomplete. The usual precautions should be observed in using the silver nitrate-ammonium hydroxide solutions.

4. The benzaldehyde treatment with micro-quantities is made in centrifuge tubes, the extraction with ether. The yield is 0.0100 g. from 0.0053 g. of acetic acid.

5. The product is a dihydrate[1] which loses water at 80–90°. Prolonged heating, at this temperature, slowly decarboxylates the acid.

C. Other Preparations

In a somewhat similar manner is prepared[2] 2-phenylbenzimidazole-2-C^{14}, m.p. 284–285° (71%). A mixture of 1.41 g. of powdered benzaldehyde-C^{14} bisulfite compound and 0.74 g. of *o*-phenylenediamine is boiled for 3 minutes in 15 ml. of alcohol. The hot filtered solution of Schiff base is diluted with 4 ml. of nitrobenzene, and the mixture is heated for a short time at 210°. After cooling, the product is collected and purified through the hydrochloride. The determination of the compound by paper chromatography is described.

The preparation of 5,6-dimethylbenzimidazole-2-C^{14} has been reported.[3]

[1]R. A. B. Copeland and A. R. Day, J. Am. Chem. Soc., *65*, 1072 (1943).

[2]D. Jerchel, H. Becker and K. Schmeiser, Z. Naturforsch, *8b*, 294 (1953); D. Jerchel, H. Fischer and M. Kracht, Ann., *575*, 162 (1952).

[3]F. Weygand and H. Simon, Z. Naturforsch., *9b*, 449 (1954).

2-HEPTADECYL-1-C^{14}-2-IMIDAZOLINE

$$C_{17}H_{35}C^*OOH \xrightarrow{CH_2N_2} C_{17}H_{35}C^*OOCH_3 \xrightarrow{NH_2CH_2CH_2NH_2}$$

$$C_{17}H_{35}C^*ONHCH_2CH_2NH_2 \xrightarrow[\Delta]{} C_{17}H_{35}-C^*(=N-CH_2-CH_2-)-NH$$

V. F. Raaen, O. K. Neville and G. A. Ropp, Atomic Energy Commission Report, ORNL-1288; Nuc. Sci. Abstracts, 6, 4759 (1952).

A. Procedure (Note 1)

Octadecanoic-1-C^{14} acid (0.283 g., 1 mmole) is esterified with ethereal diazomethane. The excess reagent is decomposed with a drop of acetic acid; then 0.7 ml. (10 mmoles) of ethylenediamine is added, the ether is distilled through a short Vigreux column, and the flask is heated at 70° for 15 hours. The excess diamine is distilled by reducing the pressure to 15 mm.; then the crystalline residue is distilled at 15–20 mm. with a bath temperature of 250°; yield 0.248 g. (80.5%), m.p. 73–77° (Note 2).

B. Notes

1. The procedure is adapted from the method of preparing substituted 2-imidazolines described by Hill[1] and modified by Morrill.[2]

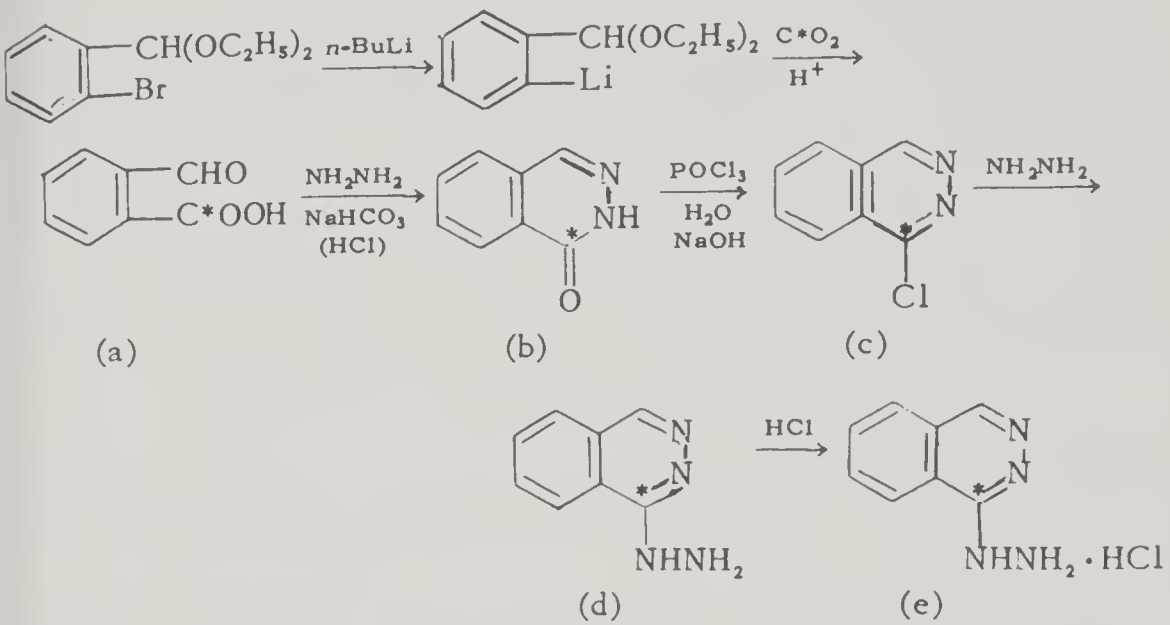
2. The melting point of the pure compound is given as 94–95° by Waldmann.[3]

[1]A. J. Hill and S. R. Aspinall, J. Am. Chem. Soc., *61*, 822 (1939).

[2]L. P. Kyrides, F. B. Zienty, G. W. Steahly and H. L. Morrill, J. Org. Chem., *12*, 577 (1947); H. L. Morrill, U. S. 2,508,415; Chem. Abstracts, *45*, 668 (1951).

[3]E. Waldmann, A. Chwala and A. Martina, Ber., *74B*, 1763 (1941).

1-HYDRAZINOPHTHALAZINE-1-C^{14} HYDROCHLORIDE
(C_1^{14}-Apresoline)



A. Murray III, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, unpublished work.

A. Procedure

(a) o-*Phthalaldehydic-C^{14} Acid* (Note 1). To a stirred solution of 4.46 mmoles of butyllithium in 20 ml. of absolute ether (Note 2) at the temperature of a Dry Ice-acetone bath, under nitrogen, is added 1.33 ml. (6.99 mmoles, 1.307 g./ml.) of *o*-bromobenzaldehyde diethyl acetal in 15 ml. of absolute ether during 7 minutes (Note 3). The cooled mixture is stirred for 40 minutes, then frozen with liquid nitrogen, evacuated, thawed with a Dry Ice-acetone bath, stirred, carbonated with 2.23 mmoles of carbon-C^{14} dioxide and hydrolyzed with 10 ml. of water containing 10 drops of saturated sodium hydroxide. The colorless alkaline solution is continuously extracted with ether for 15 hours (Note 4), then acidified with 5 ml. of concentrated hydrochloric acid and extracted with ether (28 hours). The ether solution is concentrated by evaporation (Note 5).

(b) *1(2H)-Phthalazinone-1-C^{14}* (Note 6). To the residual crude acid is added 10 ml. of water, 0.206 g. (2.45 mmoles) of sodium bicarbonate and 0.35 ml. (7.2 mmoles) of hydrazine hydrate. The mixture is refluxed for 30 minutes, then acidified by the dropwise addition of 3 ml. of concentrated hydrochloric acid and refluxed for 15 minutes. The hot solution is filtered (Note 7) and evaporated, and the residue is extracted with five 4-ml. portions of boiling chloroform. The filtered extract is evaporated

in a 25-ml. conical flask, the solid being rinsed down with solvent, which is then evaporated; yield 0.2805 g. (1.92 mmoles, 86.0% based on barium carbonate) (Note 8).

(c) *1-Chlorophthalazine-1-C*^{14} (Note 9). A mixture of the crude phthalazinone and 0.27 ml. (3 mmoles) of phosphorus oxychloride is rotated and heated under a reflux condenser on a steam-bath until solution results (5–7 minutes); then the tube is chilled with an ice-bath, and the pasty mixture is stirred (Note 10) with 4 drops of ice-water. The walls are rinsed down with 1 ml. of alcohol, the mixture is frozen with a Dry Ice-bath, an excess (4.1 ml.) of saturated ethanolic sodium hydroxide is added, and the thawing mixture is stirred, treated with an excess of Dry Ice chips and stored until the carbon dioxide has evaporated.

(d) *1-Hydrazinophthalazine-1-C*^{14} *Hydrochloride* (Note 11). The crude chlorophthalazine mixture is diluted with 10 ml. of alcohol and refluxed with 0.9 ml. (18.5 mmoles) of hydrazine hydrate for 2 hours on a steam-bath. The golden-colored solution is filtered hot with the aid of 5 ml. of wash alcohol (Note 12) and evaporated. The residue is dissolved in cold water, acidified (pH 2) with 3 ml. of concentrated hydrochloric acid and continuously extracted with dichloromethane for 26 hours. The colorless aqueous solution is basified with dilute sodium hydroxide and continuously extracted with dichloromethane for 23 hours. The extract is evaporated (Note 13), and the residue is dissolved in warm chloroform (Norit); then the solution is saturated with anhydrous hydrogen chloride, and the precipitated colorless 1-hydrazinophthalazine-1-C^{14} dihydrochloride is collected, washed with chloroform and dried at room temperature (Note 14); yield 0.2720 g. (52.3% based on barium carbonate). Purification is accomplished by dilution with 0.0979 g. of carrier and recrystallization from 3.6 ml. of 1 *N* hydrochloric acid at 0°. The dihydrochloride is dried and converted to the monohydrochloride by heating at 120° (2 hours); yield 0.2942 g., radiochemical yield 53.6% based on barium carbonate (Note 15).

B. Notes

1. The aldehyde group is blocked in alkaline media as the acetal, which is readily cleaved by the mild action of dilute acid. Also see Parham[1] and 1,2-dihydroxyanthraquinone-9-C^{14}.

2. For a description of apparatus and preparation of the butyllithium, see *p*-aminobenzoic-C^{14} acid.

3. *o*-Bromobenzaldehyde is prepared by photobromination of *o*-bromotoluene and hydrolysis of the α, α, *o*-tribromotoluene according to the procedure of Adams;[2] yield 58%, b.p. 114–115° (11.5 mm.), solid in refrig-

erator (m.p. 22°). The diethyl acetal is prepared by adaptation of the general method of Haworth;[3] yield 70%, b.p. 128–132° (10 mm.).

4. The excess starting acetal is recovered by ether extraction (5 hours) at pH 8.

5. *o*-Phthalaldehydic acid may be recrystallized from water at 0° (Norit), m.p. 96–96.5°.

6. The procedure is a modification of the method of Seekles[4] and Gabriel.[5]

7. A small amount of insoluble colorless solid is collected and washed with water; yield 2–4% by weight, m.p. 221.5–222.0° from *s*-butyl alcohol. This unidentified material is formed in high yield if the bicarbonate is omitted and hydrazine hydrochloride and alcohol-water are used.

8. 1(2*H*)-Phthalazinone melts at 183–184°; yield 95–98%.

9. The procedure is a modification of that of Druey,[6] Vaughan[7] and Gabriel.[5] Pure 1-chlorophthalazine melts at 113°.

10. The short glass rod is left in the reaction flask.

11. The procedure is a modification of that of Druey.[6]

12. The orange-colored, water-insoluble precipitate is 1,2-bis(1-phthalazinyl-1-C^{14})hydrazine; yield 0.001–0.003 g., m.p. 273–276° (dec.) from dioxane.

13. Purification of the free base may also be accomplished by vacuum sublimation at 85–95° (10 μ), or recrystallization from methanol, m.p. 177.5–178° (dec.)

14. A sample of the dihydrochloride dried under vacuum at room temperature melted at 275–276° (dec.) when immersed in a bath initially at 260°. A sample of the hydrochloride prepared by crystallizing the free base from 2 *N* hydrochloric acid[6] melted at 273–274° (dec.) when immersed in a bath at 260°. The dihydrochloride is stable at 80°, but is converted to the monohydrochloride by heating at 120°.

15. In trial runs the yield was 56–70% based on *o*-phthaladehydic acid at the 2-mmole level.

[1]W. E. Parham and E. L. Anderson, J. Am. Chem. Soc., *70*, 4187 (1948).

[2]R. Adams and E. H. Vollweiler, J. Am. Chem. Soc., *40*, 1732 (1918); *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 89.

[3]R. D. Haworth and A. Lapworth, J. Chem. Soc., *1922*, 76.

[4]L. Seekles, Rec. trav. chim., *43*, 328 (1924).

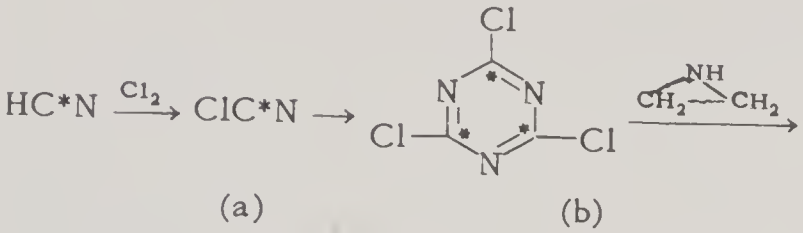
[5]S. Gabriel and A. Neumann, Ber., *26*, 521 (1893).

[6]J. Druey and B. A. Ringier, Helv. Chim. Acta, *34*, 195 (1951); M. Hartmann and J. Druey, U. S. 2,484,029; Chem. Abstracts, *44*, 4046 (1950).

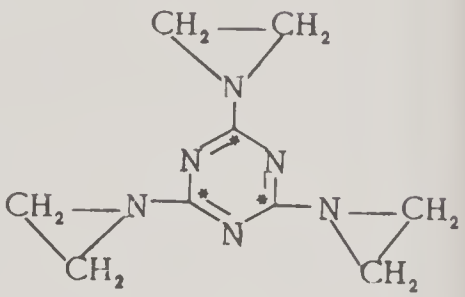
[7]W. R. Vaughan and S. L. Baird, Jr., J. Am. Chem. Soc., *68*, 1314 (1946).

2,4,6-TRIS(1-AZIRIDINYL)-*s*-TRIAZINE-C_3^{14} (Triethylenemelamine-C_3^{14})

METHOD I



(a) (b)



(c)

D. L. Williams and A. R. Ronzio, Atomic Energy Commission Report, AECU-1232; Nuc. Sci. Abstracts, 5, 3393 (1951).

A. Procedure

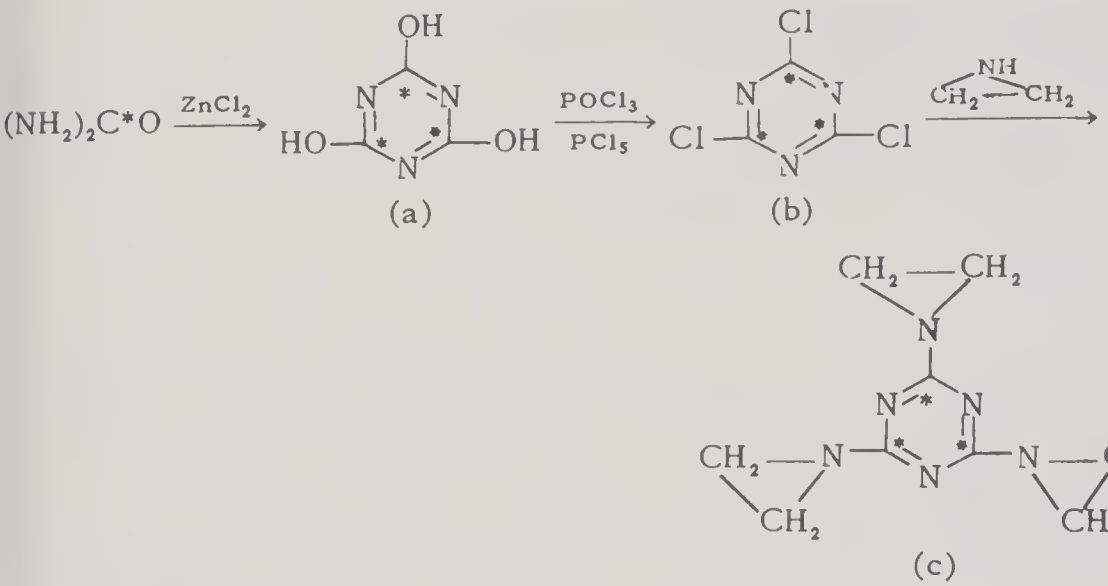
(a) *Cyanogen-C^{14} Chloride* (Note 1). To an anhydrous mixture (Note 2) of 0.015 g. of chloroform and 2.78 mmoles of chlorine, frozen in an evacuated flask cooled with liquid nitrogen, is added by distillation the dried hydrogen cyanide-C^{14} liberated from 2.6 mmoles of sodium cyanide-C^{14} (Note 3). The liquid nitrogen-bath around the mixture is replaced by a Dry Ice-trichloroethylene-bath at -40°, which is then allowed to warm to 0° over a period of 16 hours. The chlorine reacts almost completely.

(b) *Cyanuric-C_3^{14} Chloride* (Note 4). To the reaction mixture of cyanogen-C^{14} chloride and anhydrous hydrogen chloride (Note 5), cooled with liquid nitrogen, is added 0.15 ml. of purified dioxane by distillation through phosphorus pentoxide. The flask is closed, and the mixture is stored at room temperature for 168 hours. The solvents, hydrogen chloride and any unreacted starting material are removed by vacuum distillation, leaving 0.1822 g. of colorless crystalline residue (Note 6).

(c) *2,4,6-Tris(1-aziridinyl)-s-triazine-C_3^{14}* (Note 7). To a stirred mixture of 0.3 g. (2.97 mmoles) of triethylamine, 0.129 g. (2.97 mmoles) of ethylenimine and 3 ml. of benzene at –2° is added dropwise during 15 minutes a solution of the crude cyanuric-C_3^{14} chloride in 6 ml. of dry benzene (Note 8). The mixture is stirred for 1 hour, then filtered, and the precipitate is washed with several portions of benzene. The combined filtrate and washings are evaporated to dryness at 40° under vacuum;

the colorless residue weighs 0.2068 g. Purification (Note 9) is accomplished by extraction with twenty 10-ml. portions of absolute ether, followed by evaporation of the solvent. The yield is 0.1696 g. (95.8% based on sodium cyanide); the compound exhibits a change in appearance at 150–160° (Note 10). The molar specific activity is in agreement with three times that of the sodium cyanide.

METHOD II



M. V. Nadkarni and C. L. Brown, Department of Pharmacology, The George Washington University School of Medicine, Washington, D. C., private communication; M. V. Nadkarni, E. I. Goldenthal and P. K. Smith, Cancer Research, *14*, 559 (1954).

A. Procedure

(a) *Cyanuric-C_3^{14} Acid*, (*s-Triazine-2,4,6-triol-C_3^{14}*) (Note 11). A mixture of 0.120 g. of dry urea-C^{14} and 0.240 g. of powdered, freshly-fused, anhydrous zinc chloride, under an atmosphere of dry nitrogen in a side arm test tube, is heated in an oil-bath at 220° for 1.5 hours. The mixture is cooled to room temperature, treated with 1.5 ml. of 0.1 *N* hydrochloric acid and stored overnight in a refrigerator. The colorless product is collected on a filter, washed with cold acidified water and dried at 90°; yield 0.056 g., 65% (Note 12).

(b) *Cyanuric-C_3^{14} Chloride* (Note 13). To 0.056 g. of cyanuric-C_3^{14} acid, in a long narrow test tube closed with a phosphorus pentoxide drying tube, is added a mixture of 0.5 ml. of phosphorus oxychloride and 0.30 g. of phosphorus pentachloride. The mixture is refluxed at 150–155° for 2 hours (Note 14), then cooled in a bath at –60°, and the excess phosphorus halides are slowly decomposed by adding small amounts of crushed ice (Note 15). The mixture is made just alkaline to litmus by

the addition of sodium carbonate in the cold. The solution of cyanuric-C_3^{14} chloride may be used directly, or the product may be isolated by ether extraction, m.p. 146°.

(c) *2,4,6-Tris(1-aziridinyl)-s-triazene-*C_3^{14} (Note 16). To the stirred solution of cyanuric-C_3^{14} chloride maintained at 0–5° is added 0.1 g. of sodium carbonate, followed by the dropwise addition over a period of 30 minutes of an ice cold solution of 0.3 ml. of ethylenimine in 2 ml. of saturated sodium carbonate solution. The mixture is stirred for 1 hour at room temperature, then extracted with several portions of chloroform. Evaporation of the solvent leaves the crude product, which is recrystallized from ether (Note 17); yield 0.029 g. (22%), m.p. 150–152° (Note 18). The molar specific activity agrees with three times that of the urea. The purity is confirmed by radioautography and paper chromatography with two binary solvent systems (Note 19).

B. Notes

1. The procedure is adapted from the method suggested by Hartley.[1]

2. The compounds are dried by vacuum distillation through a tube containing phosphorus pentoxide-on-glass wool into a flask cooled with liquid nitrogen.

3. Hydrogen cyanide, generated *in vacuo* by addition of 1:1 sulfuric acid to the salt, is distilled with liquid nitrogen cooling through a trap at −25° to −30°, and through phosphorus pentoxide. The use of concentrated sulfuric acid[2] leads to the formation of carbon monoxide.

4. The procedure is adapted from the method of Oldham.[3]

5. The hydrogen chloride exerts a catalytic effect upon the polymerization of cyanogen chloride.

6. Oldham has reported a yield of 80 g., based on 95 g. of cyanogen chloride; m.p. 146°.

7. The procedure is essentially that described by Bestian.[4] The ethylenimine may be prepared by the procedure of Leighton.[5]

8. A colorless precipitate of triethylamine hydrochloride begins to form at once. A two-necked flask equipped with a mercury-sealed stirrer and drying tube is used. The addition tube is rinsed with 2.5 ml. of benzene.

9. The crude product weighs 16% in excess of the theoretical yield. The choice of purification solvents is limited by the fact that in solution triethylenemelamine undergoes a rapid change at 50°. The compound is only moderately soluble in ethyl acetate,[4] which seems to dissolve some triethylamine hydrochloride as well.

10. In a preheated bath at 148°, the compound melts to a clear liquid which quickly polymerizes. Further purification may be accomplished by dissolution in a minimal volume of dry benzene at 50° (0.1424 g. per

13 ml.) and filtration at room temperature through a bed of Norit on Celite, washing with 2 ml. of benzene. The filtrate is diluted at 50° with 15 ml. of hexane and stored at 0° for at least 2 hours. The crystalline product is collected, washed with 2 ml. of hexane and dried under vacuum to constant weight; recovery 77–82%.

11. The procedure is an adaptation of the method of von Walther.[6]

12. The dihydrate is efflorescent, and the anhydrous compound decomposes without melting, evolving cyanic acid.

13. The procedure is adapted from the method of Beilstein.[7]

14. A clear yellowish solution forms in about 40 minutes.

15. Violent reaction must be avoided, and the temperature must be kept below 5°.

16. The procedure is an adaptation of the method of Wystrach and Kaiser.[8]

17. From the standpoint of stability, the product is stored preferably in chloroform solution in a refrigerator, with evaporation of aliquots as needed.

18. The ultraviolet spectral properties agree with those of an authentic sample.

19. A descending (Whatman No. 1) paper strip chromatogram using a system of 1-butanol saturated with 1% ammonia solution shows a single peak of radioactivity at R_f 0.64, while an acetone-water (3:2 v/v) system also shows a single peak at R_f 0.85.

C. Other Preparations

The preparation of cyanogen-C^{11} chloride has been described by Rice and Yankwich.[9] Silver cyanide-C^{11} was precipitated by treating a solution of 1 mmole of sodium cyanide-C^{11} (buffered with sodium bicarbonate) with excess silver nitrate. The carbonate was destroyed with 0.1 *N* nitric acid, and the moist precipitate, collected with Celite, was treated with 2 mmoles of chlorine (frozen) in a sealed tube at −5° (10 minutes). The mixture was distilled through phosphorus pentoxide, then treated with partially amalgamated copper turnings to remove excess chlorine; yield over 80% based on silver cyanide. Williams and Ronzio have reported stoppage of the reaction by the coating of silver cyanide by silver chloride and, also, reduction of the yield by reaction of the product with silver cyanide, forming cyanogen.

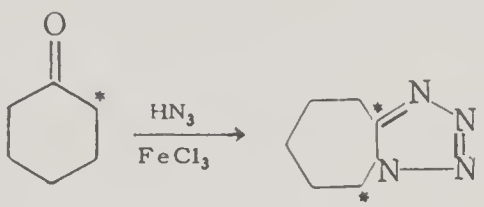
The preparation of cyanuric chloride from moist sodium cyanide and chlorine has been adapted satisfactorily to a micro-scale by Williams and Ronzio.

[1]W. N. Hartley, J. J. Dobbie, and G. D. Lauder, J. Chem. Soc., 79, 852 (1901).

[2]J. Wade and L. C. Panting, *ibid.*, 73, 255 (1898).

[3]W. N. Oldham, U. S. 2,417,659; through Chem. Abstracts, *41*, 3494 (1947).
[4]H. Bestian, Ann., *566*, 210 (1950).
[5]P. A. Leighton, W. A. Perkins and M. L. Renquist, J. Am. Chem. Soc., 69, 1540 (1947).
[6]R. von Walther, J. prakt. Chem., (2) 79, 126 (1909).
[7]F. Beilstein, Ann., *116*, 357 (1860).
[8]W. P. Wystrach and D. W. Kaiser, U.S. 2,520,619; Chem. Abstracts, *45*, 8049, (1951).
[9]M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, *1949*, p. 158.

6,7,8,9-TETRAHYDRO-5*H*-AZEPOTETRAZOLE-5,9a-$C^{14}_{1/2}$ (C^{14}_1-Metrazole)



A. Murray III and A. R. Ronzio, Atomic Energy Commission Report, AECU-1882; Nuc. Sci. Abstracts, 6, 2054 (1952).

A. Procedure (Note 1)

To a stirred, ice-cooled mixture (Note 2) of 0.25 g. of freshly prepared anhydrous ferric chloride catalyst (Note 3) and 3.65 ml. of dry benzene-hexane solution (85:15 v/v) containing 0.383 g. (8.9 mmoles) of hydrazoic acid (Note 4) is added slowly a solution containing 0.152 g. (1.55 mmoles) of cyclohexanone-2-C^{14}. The solution of the ketone in 3.8 ml. of the benzene-hexane solvent is added from an addition tube: 1 ml. within 1 minute (Note 5), and the remainder jet-wise every 15 seconds during 10 minutes. The mixture is stirred overnight, at room temperature, then made basic with 1.2 g. of sodium hydroxide. The thick suspension is extracted continuously with benzene for 24 hours, and the extract is evaporated to dryness in the base of a vacuum sublimator (Note 6). Sublimation at 80–110° (10 μ) gives the crude product (m.p. 52–55°) in 96.5–98.8% yield based on four trial runs. Purification is achieved by dissolution in water, treatment with carbon to remove a trace of insoluble oil, and evaporation of the filtrate (Note 7). Two recrystallizations from isopropyl ether at –25° (Note 8) give a product melting at 56–58° (Note 9).

B. Notes

1. The procedure is a modification of the method of Chapman.[1] Since concentrated hydrazoic acid is both dangerously explosive and highly toxic, the preparation is carried out behind a safety screen in a well ventilated hood.

2. As nitrogen is evolved, the mixture is stirred under a reflux condenser protected by a drying tube.

3. Anhydrous ferric chloride is prepared by passing chlorine over powdered iron in a tube heated with a burner. The sublimed product is collected in a bulb cooled with water and swept with dry carbon dioxide before being opened.

4. Directions are given by Chapman[1] for preparing the hydrazoic acid solution from sodium azide; an excellent review of the subject is presented by Audrieth.[2] The acid solution is assayed by titrating an aliquot with standard sodium nitrite solution (ferric chloride indicator) according to the procedure of Reith and Bouwman.[3]

5. Since hydrazoic acid reacts slowly with benzene in the presence of the catalyst, but more rapidly with cyclohexanone, the initial addition of ketone is made without delay.

6. The residue in three trial runs was equivalent to 111, 101 and 106% of theory.

7. A blank run without cyclohexanone gave rise to a similar trace of water-insoluble oil.

8. The filtration is done by means of a filter stick, while the mixture is kept in a cooling bath. The solubility of metrazole (mg./ml.) in several ethers was found to be: ethyl 61.2 (28°), 2.36 (−78°); isopropyl 10.6 (0°), 1.24 (−78°); butyl 3.87 (0°), 1.30 (−25°).

9. The pure compound melts at 59.5–60.5°. Metrazole forms an insoluble addition compound (m.p. 170–173°) when treated with an excess of aqueous mercuric chloride. The complex may be collected and decomposed with hydrochloric acid, and the metrazole is recovered (88–96%) by benzene extraction.

[1]N. B. Chapman, H. McCombie and B. C. Saunders, J. Chem. Soc., *1945*, 929; *Organic Reactions*, Vol. III, Wiley, New York, 1946, p. 318.

[2]L. F. Audrieth, Chem. Rev., *15*, 169 (1934).

[3]J. F. Reith and J. H. A. Bouwman, Pharm. Weekblad, *67*, 475 (1930); Chem. Abstracts, *24*, 3968 (1930).

HYDROCARBONS

METHANE-C*

METHOD I

$$C^*O_2 \xrightarrow[\text{Cat.}]{H_2} C^*H_4$$

G. R. Hennig, Atomic Energy Commission Report, AECD-1794.

A. Procedure

Methane-C^{14}. A mixture of 2 l. of carbon-C^{14} dioxide at 60–80 mm. pressure and hydrogen at 4–5 times that pressure is passed repeatedly, by Toepler pump (Figure IX, 1) through 2 g. of ruthenium powder contact catalyst[1] at 200–300° (Note 1). The water formed is condensed in a trap cooled with a Dry Ice-acetone bath. The end of the reaction is determined by the absence of formation of a ring of condensed carbon dioxide when a second trap is cooled with liquid nitrogen. When the reaction is complete (2 hours), the gas mixture is exposed to a palladium thimble maintained at 450° to remove the excess hydrogen. The separation is facilitated by partially condensing the product in a trap cooled with liquid nitrogen. The yield is quantitative.

METHOD II

$$C^*H_3I \xrightarrow{Mg} C^*H_3MgI \xrightarrow{H^+} C^*H_4$$

C. D. Wagner, D. P. Stevenson and J. W. Otvos, J. Am. Chem. Soc., 72, 5786 (1950).

A. Procedure

Methane-C^{13}. A Grignard solution is prepared from 0.8 g. of magnesium turnings, 1.98 g. of methyl-C^{13} iodide and 30 ml. of absolute ether in a

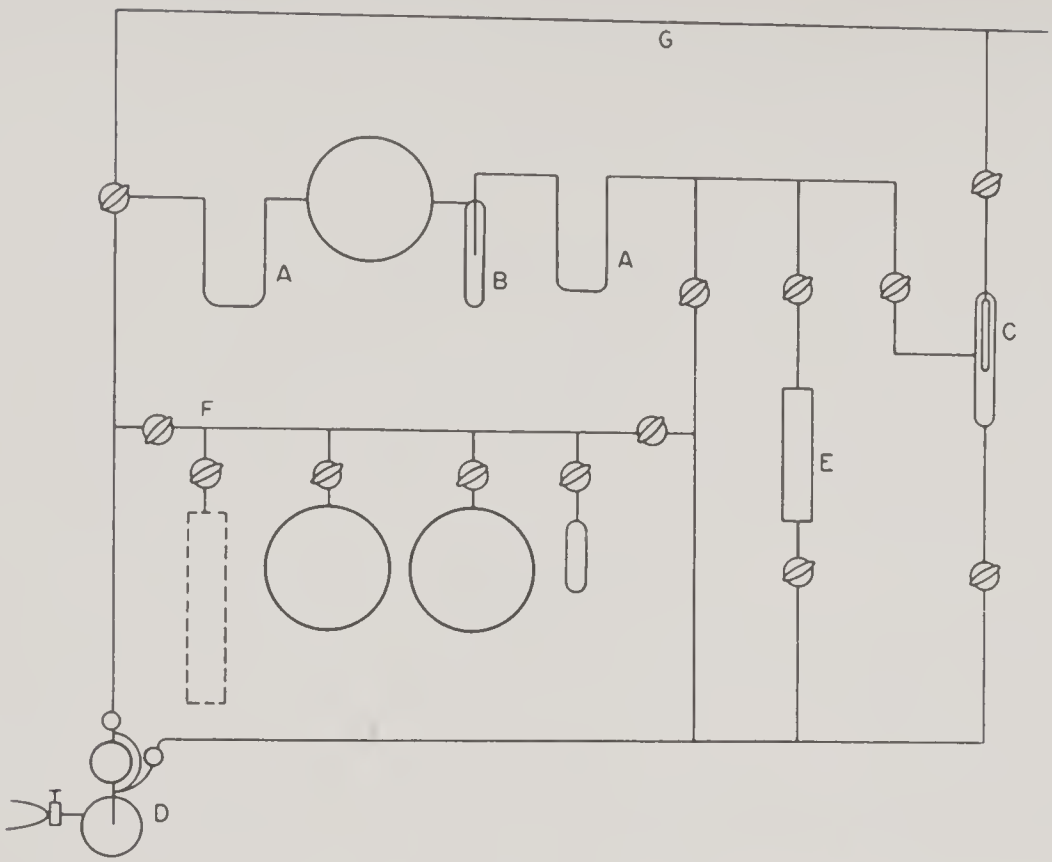


Fig. IX, 1. Apparatus for preparation of methane (G. R. Hennig). A, U-trap; B, catalyst; C, palladium valve; D, Toepler pump; E, Quartz, copper oxide furnace; F, counter filling manifold: G, vacuum manifold.

500-ml. flask. The flask is attached to a vacuum manifold, and the mixture is frozen in liquid air and outgassed. The evacuated flask is disconnected from the line, then cooled in an ice-bath, and the complex is hydrolyzed by the cautious addition of 5 ml. of water through the stopcock. The mixture is frozen with liquid air, and the spectrometrically pure product is transferred into a storage bulb by means of a Toepler pump; yield 12 mmoles, 86%.

METHOD III

$$C^*H_3I \xrightarrow{Li} C^*H_3Li \xrightarrow{H_2O} C^*H_4$$

E. Müller and W. Rundel, Ber., *88*, 917 (1955).

A. Procedure

(a) *Methyllithium-C^{14}* (Note 2). Into a flask cooled with liquid nitrogen and containing 0.5 g. of lithium chips and 35 ml. of absolute ether is vacuum-distilled 3.83 g. (27 mmoles) of methyl-C^{14} iodide. The closed flask is removed from the vacuum line and cooled with an ice-bath until reaction is nearly complete; then the mixture is warmed in a water-bath

for 1 hour. The solution is filtered under nitrogen (Note 3) into a supply burette; yield 31 ml., 20.8 mmoles (77%).

(b) *Methane-C^{14}*. To a mixture of 20 ml. of ether, 0.2 ml. of water and 0.4 ml. of alcohol under nitrogen is added dropwise 14 ml. (9.5 mmoles) of methyllithium-C^{14} solution. The methane-C^{14} is passed through a reflux condenser and a spiral trap cooled with acetone-Dry Ice to remove ether vapor (Note 4). The system is swept with nitrogen until a total of 400 ml. of gas is collected over saturated salt solution. The hydrolysis mixture is titrated with 0.1 *N* hydrochloric acid to determine the methyllithium upon which yield is based (Note 5).

B. Notes

1. The catalyst is prepared by boiling ruthenium with 6 *N* nitric acid, washing, and heating in air at 400° for 30 minutes.

2. For other preparations of methyllithium-C^{14}, see index. The procedure is a modification of that of Gilman.[2] Reaction in refluxing ether, or in a sealed tube without cooling, gave yields of only 30%. The chief side reaction is the Wurtz coupling. Methyllithium, unlike butyllithium, is stable for weeks in ether.[2]

3. The reaction flask is fitted with a sealed-in frit.

4. Trial experiments have shown that all but 0.7 mole per cent of ether is removed from the gas stream.

5. The formation of methane-C^{14} by decomposition of methyllithium-C^{14} with diazomethane has been described by Müller and Rundel; yield 97%. The preparation was incidental to a study of the hydrogen-metal exchange reaction.

[1]F. Fischer, T. Bahr and A. Meusel, Breenstoff-Chem., *16*, 466 (1935); through Chem. Abstracts, *30*, 3967 (1936).

[2]H. Gilman, E. A. Zoellner and W. M. Selby, J. Am. Chem. Soc., *55*, 1252 (1933).

PROPANE-1-C^{13}

$$CH_3CH_2C^*OOH \xrightarrow{LiAlH_4} \underset{(a)}{CH_3CH_2C^*H_2OH} \xrightarrow{I_2} \underset{(b)}{CH_3CH_2C^*H_2I} \xrightarrow{Zn-Cu} \underset{(c)}{CH_3CH_2C^*H_3}$$

A. S. Gordon and S. Heimel, J. Am. Chem. Soc., 73, 2942 (1951).

A. Procedure

(a) *1-Propanol-1-C^{13}* (Note 1). A mixture of 44 mmoles of sodium propionate-1-C^{13} and 20 ml. of 2-ethylhexanoic acid (b.p. 226°) is distilled slowly through a fractionating column until about 1 ml. of the high-boiling acid and the propionic-1-C^{13} acid have been collected in a receiver containing 15 ml. of hexyl ether. To a stirred slurry of 2.4 g. of lithium aluminum hydride and 75 ml. of hexyl ether, cooled in an ice-bath, is added slowly the acid solution, followed by 10 ml. of the ether. The mixture is heated at 75–80° for 30 minutes, then cooled and hydrolyzed by the slow addition of 40 ml. of undecanol. The product and about 1 ml. of the high-boiling alcohol are collected by distillation through the fractionating column.

(b) *1-Iodopropane-1-C^{13}*, (*Propyl-1-C^{13} Iodide*). To a mixture of the crude 1-propanol-1-C^{13} and 0.71 g. of red phosphorus is added 8.6 g. of iodine, in small portions with ice-bath cooling. The mixture is stored overnight at room temperature and then heated, under a low-temperature reflux condenser, for 1.5 hours in a boiling water-bath. The product is vacuum-distilled through a thin layer of potassium hydroxide into a receiver cooled with liquid nitrogen (Note 2).

(c) *Propane-1-C^{13}* (Note 3). A mixture of the crude 1-iodopropane-1-C^{13}, 10 ml. of hexanol and 2 drops of 0.2 *N* sulfuric is frozen, with a liquid nitrogen-bath, in a flask equipped with a low-temperature condenser (cooled by Dry Ice) and an addition tube (Note 4) containing 4 g. of zinc-copper couple.[1] The system is evacuated and closed off from the vacuum manifold. To the mixture at room temperature is added the zinc-copper couple, warming with a water-bath being necessary only at the end of the reduction. The crude product is allowed to expand into a large evacuated vessel and then is condensed into a small bulb cooled by liquid nitrogen, any noncondensible gases being removed by pumping. Moisture and hexane are removed by distilling the product under vacuum at −78°, and further purification is effected by repeatedly passing the product over fuming sulfuric acid and potassium hydroxide. The yield is 71% based on sodium propionate; the purity (trial runs) is 98–99.3% (Note 5).

B. Notes

1. The procedure is a modification of the method of Nystrom and Brown.[2] For other isotopic procedures see Fries and Calvin (1-propanol-1-C^{14}) and Adams, Selff and Tolbert (1-bromopropane-1-C^{14}).

2. The crude product is contaminated with iodine and hydrogen iodide. The purification train described in the preparation of methyl-C^{14} iodide by Method I effectively removes hydrogen iodide; also see propyl-1-C^{14} iodide.

3. The method is a modification of the method of Maass and Wright.[3]

4. The side arm with a 90° bend is connected to the flask with a lubricated ground-glass joint.

5. The impurities are shown by the mass spectrometer to be ethane, butane, propylene and butylene. The ethane may be reduced to negligible amounts by twice freezing the product with a mush of technical pentane (−140°) and pumping.

6. Fractional distillation is avoided by converting the alcohol to the less volatile benzoate, with benzoyl chloride and pyridine, then removing the ether and hydrolyzing the benzoate in constant-boiling hydrobromic acid.

C. Other Preparations

1-Propanol-1-C^{14} and 1-propanol-2-C^{14} have been prepared by Kummer[4] in the described manner from the appropriate acids. The constant-boiling alcohol-water mixtures were dehydrated with calcium carbide, and the anhydrous alcohol was redistilled.

Propane has been prepared in 70% yield by electroreduction of acetone in sulfuric acid solution, using electrodes of zinc amalgam and platinum. The product, as prepared by Gordon,[5] was 99.5% pure, propylene being the only impurity. 2-Propanone-2-C^{13} would form propane-2-C^{13}.

Propane-1-C^{13} has been prepared by Wagner[6] in 26% yield from 3.2 g. of potassium cyanide-C^{13}. Propionitrile-1-C^{13} (80%), formed by condensation of cyanide-C^{13} with ethyl bromide, was hydrolyzed with dodecyl alcohol and hydrochloric acid to form dodecyl propionate-1-C^{13} (75%), which was then reduced to 1-propanol-1-C^{13} (75%) by reduction with lithium aluminum hydride in ether solution. Propyl-1-C^{13} bromide (90%) was prepared from the alcohol (Note 6) by heating with hydrobromic acid, then was reduced to the hydrocarbon (65%) by hydrolysis of the Grignard reagent. Traces of olefin impurity were removed by treatment with aqueous bromine (5 minutes at room temperature in the dark). Methyl-C^{13} bromide and excess bromine were removed by shaking with ethanolamine at room temperature, then for 3 hours at 100° in a sealed tube. Ammonia was removed by passage through powdered silver nitrate. The product was redistilled through a Podbielniak Heli-Grid column at −43.5° to −39°.

Butane-1-C^{13} has been prepared in 17% yield[6] from potassium cyanide-C^{13} by a similar reaction sequence, the intermediates being: butyronitrile-C^{13}, octyl butyrate-1-C^{13}, 1-butanol-1-C^{13} and 1-bromobutane-1-C^{13}.

Butane-1-C^{14} has been prepared by Wagner,[6] by hydrogenation of 1-butene-4-C^{14} over a nickel catalyst at 100°; see 2-methylpropane-1-C^{13}. The crude yield was 98%, but the product contained about 10% each of hexane and propane arising from allyl radicals in the preparation of the olefin; over-all yield 61% based on methanol-C^{14}. The impurities were

removed by slow fractional distillation at −78°. The preparation of ethane-C_2^{14} (yield "considerable") by reduction of acetylene-C_2^{14} over nickel at room temperature (5–10 minutes) has been reported by Keyer.[9]

The intramolecular isomerization of propane-1-C^{13} to propane-1,2-$C^{13}_{1/2}$, by the action of water-promoted, aluminum bromide catalyst at 25°, has been described by Beek.[7]

The preparation of propyl-1-C^{13} iodide, by treating 1-propanol-1-C^{13} with constant-boiling hydriodic acid, has been reported by Sakami.[8]

[1]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 185, method (a).
[2]R. F. Nystrom and W. G. Brown, J. Am. Chem. Soc., *69*, 2548 (1947).
[3]O. Maass and C. H. Wright, J. Am. Chem. Soc., *43*, 1098 (1921).
[4]J. T. Kummer and P. H. Emmett, J. Am. Chem. Soc., *75*, 5177 (1953).
[5]A. S. Gordon and S. Heimel, J. Am. Chem. Soc., *73*, 2942 (1951).
[6]C. D. Wagner, D. P. Stevenson and J. W. Otvos, *ibid.*, *72*, 5786 (1950).
[7]O. Beek, J. W. Otvos, D. P. Stevenson and C. D. Wagner, J. Chem. Phys., *16*, 255 (1948).
[8]M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 202.
[9]N. P. Keyer, B. V. Klimenak and G. V. Isagulyants, Doklady Akad. Nauk S.S.S.R., *85*, 1029 (1952); Nuc. Sci. Abstracts, *7*, 457 (1953); AEC-tr-1825.

PROPENE-1-C^{14}

$$CH_3CH_2C^*OOH \xrightarrow[H_2SO_4]{CH_3CH_2CH_2OH} CH_3CH_2C^*OOCH_2CH_2CH_3 \xrightarrow[CuCr_2O_4]{H_2}$$

(a)

$$CH_3CH_2C^*H_2OH \xrightarrow[P]{Br_2} CH_3CH_2C^*H_2Br \xrightarrow{(CH_3)_3N}$$

(b) (c)

$$CH_3CH_2C^*H_2\overset{+}{N}(CH_3)_3\,Br^- \xrightarrow{Ag_2O} CH_3CH_2C^*H_2\overset{+}{N}(CH_3)_3\,OH^- \xrightarrow{-\Delta}$$

(d)

$$CH_3CH{=}C^*H_2$$

(e)

B. A. Fries and M. Calvin, J. Am. Chem. Soc., *70*, 2235 (1948).

A. Procedure

(a) *Propyl Propionate-1-C^{14}* (Note 1). Sodium propionate-1-C^{14} (40 mmoles) is refluxed for several hours with a mixture of 9.0 ml. of propyl alcohol (Note 2), 10.0 ml. of benzene and 1.1 ml. of sulfuric acid. The

esterification is driven to completion by azeotropic distillation (Note 3). The flask is cooled when the distillation temperature reaches 74° (Note 4); then the sulfuric acid is destroyed by addition of 1 g. of calcium carbonate. The distillation is continued and again stopped when the temperature reaches 92° (Note 5). The remaining ester-alcohol solution is separated from residual solids by vacuum distillation.

(b) *1-Propanol-1-C^{14}* (Notes 1 and 6). The ester-alcohol solution (8 ml.) is transferred to a high-pressure hydrogenation bomb containing 2.0 g. of Adkins copper chromite catalyst (Note 7). The bomb is charged with hydrogen (2500 p.s.i.) and heated to 250° for 9 hours. The contents of the bomb are transferred, on the vacuum line, into a flask containing calcium oxide. The dried alcohol is vacuum-transferred into a small distilling flask and fractionated, using the column described in Note 3. The fraction boiling at 96–97° is collected, and the forerun (2 ml.) is returned to the distilling flask with 2.5 ml. of carrier propyl alcohol. This mixture is distilled, and the same procedure is again repeated with 2.0 ml. of carrier. The combined yield is 10.3 ml., b.p. 96–97°. The radiochemical yield is about 84% based on the acid (Note 8).

(c) *1-Bromopropane-1-C^{14}*, (*Propyl-1-C^{14} Bromide*). The bromide is prepared from the alcohol by the phosphorus and bromine method of Gosborn, Boyd and Degering,[1] but with the use of additional phosphorus and bromine; yield 76%.

(d) *Trimethylpropyl-1-C^{14}-ammonium Hydroxide.* Propyl-1-C^{14} bromide (5 ml.) is refluxed for several hours with an excess of trimethylamine in alcohol solution. The solvent is evaporated, and the salt is dissolved in water and treated with an excess of freshly prepared silver oxide to form the free base. Silver bromide and excess silver oxide are removed by filtration.

(e) *Propene-1-C^{14}*. The quaternary hydroxide is pyrolyzed by boiling the aqueous solution to dryness in a stream of nitrogen in a system consisting of a cold-water condenser; a gas-washing bottle, containing *N* hydrochloric acid to remove trimethylamine; Drierite; and a liquid nitrogen trap to collect product. The yield is approximately 90% based on the propyl bromide, the purity is 99% (Note 9), and degradation studies indicate that there is no isotopic rearrangement (Note 10).

B. Notes

1. The method is that of Sakami[2] for preparing propyl propionate-1-C^{13} and 1-propanol-1-C^{13}.

2. The use of this ester dilutes the C^{14}-activity, but eliminates the difficult separation of two alcohols following hydrogenolysis.

3. A 16-inch, 6-mm. (i.d.), vacuum-jacketed, unpacked fractionating column is used.

4. Water is removed as the ternary azeotrope (b.p. 68.5°) with propyl alcohol and benzene.

5. The remaining benzene is removed as the binary azeotrope (b.p. 77.1°) with propyl alcohol.

6. For other isotopic procedures, see Gordon and Heimel (1-propanol-1-C^{13}) and Adams, Selff and Tolbert (1-propanol-1-C^{14}).

7. Aminco, 43-ml. size. For the catalyst, see 1-propanol-1-C^{14}, Note 5 (Adams, Selff and Tolbert).

8. Trial experiments gave yields of 95% for the esterification and hydrogenation, as measured by saponification.

9. Analysis shows 0.5% butenes, 0.5% ethylene, and a product that is 97–100% unrearranged.

A side reaction of this pyrolysis[3] forms small amounts of methanol and a mixed tertiary amine. The method is a good general preparative one, as the yield of olefins is at least 70%.

10. Directions are given by Fries for oxidizing the olefin at room temperature with potassium permanganate at pH 4, forming acetic acid (70%), carbon-C^{14} dioxide (90%), and some oxalate-C_1^{14}, originating from C-1 and C-2. The propene-1-C^{14} was 97–100% unrearranged.

C. Other Preparations

Fries[4] has studied the preparation of propene-1-C^{14} by several other methods. Dehydration of 1-propanol-1-C^{14} with metaphosphoric acid, or with aluminum oxide, resulted in 50% and 35% isomerization, respectively, to propene-3-C^{14}, as determined by acid permanganate degradation. The yield of propene-1,3-$C_{1/2}^{14}$ was 40% and 95%, and the purity was 80% and 97%, respectively, by mass spectrometric analysis.

The preparation of a number of esters by a similar procedure has been reported by Andrianova.[5] The C_1^{14}-acid was mixed with concentrated sulfuric acid and then with the alcohol. The mixture was stored for 1–2 days at room temperature, then treated twice with saturated sodium chloride solution (volume twice that of the alcohol), the upper layer being separated, dried over sodium sulfate, weighed and distilled; see table below:

Ester	% Radiochemical yield	ml. Alcohol/ g. Acid	ml. H_2SO_4
Ethyl acetate-1-C^{14}	98	6.0/1.956	1.7
Ethyl 3-methylbutyrate-1-C^{14}	83	5.8/2.045	1.6
Propyl propionate-1-C^{14}	67	5.0/1.685	1.3

A similar preparation of ethyl valerate-1-C^{14} has been described by Lukovnikov;[6] yield 70%, b.p. 143–147°.

The preparation of propene-2-C^{14} has been reported by Neyman;[7] radiochemical yield 3% based on barium carbonate. 2-Propanone-2-C^{14} was hydrogenated over nickel at 20° and 60 atmospheres pressure, then the 2-propanol-2-C^{14} was dehydrated over aluminum oxide at 400°.

The preparation of C_1^{14}-propene (radiochemical yield 81%) has been described (diagram) by Andrianova.[8] 1-Propanol-1-C^{14} (5.0 ml.) was passed dropwise from a dropping funnel through a 10 × 220 mm. bed of granulated aluminum oxide maintained at 380–385°. The gaseous product was passed through a water-cooled condenser and receiver, and collected in a gasometer over sodium chloride solution; yield 1205 ml. (S.P.T). The yield in trial preparations was not less than 97% of unsaturated compounds, 91% propene and 0.6% aldehydes.

C_1^{14}-2-Methylpropene has been prepared[8] in the same manner by dehydration of 2-methyl-1-propanol-1-C^{14} over aluminum oxide at 415–420°; radiochemical yield 90%.

[1]*Organic Syntheses,* Coll. Vol. I, Wiley, New York, 1941, p. 37.

[2]M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon,* Wiley, New York, 1949, p. 202.

[3]J. von Braun, Ann., *382,* 1 (1911); W. Hanhart and C. K. Ingold, J. Chem. Soc., *1927,* 1014; C. K. Ingold and C. C. N. Vass, J. Chem. Soc., *1928,* 3125.

[4]B. A. Fries and M. Calvin, J. Am. Chem. Soc., *70,* 2235 (1948).

[5]T. I. Andrianova and E. A. Andreev, Doklady Akad. Nauk U.S.S.R., *86,* 945 (1952); Nuc. Sci. Abstracts, *7,* 4098 (1952); AEC-tr-1826.

[6]A. F. Lukovnikov, M. B. Neyman, A. A. Bag, L. M. Rodionova, I. S. Samoukina and N. V. Bliznyak; AEC-tr-1829.

[7]M. B. Neyman, A. F. Lukovnikov and B. Z. Iofa, Doklady Akad. Nauk S.S.S.R., *78,* 493 (1951); Chem. Abstracts, *46,* 412 (1952).

[8]T. I. Andrianova and E. A. Andreev, Doklady Akad. Nauk, S.S.S.R., *87,* 45 (1952); AEC-tr-1823.

2-METHYLPROPANE-1-C^{13}

$$(CH_3)_2CO \xrightarrow{C^*H_3MgI} C^*H_3C(CH_3)_2 \quad | \quad OMgI \xrightarrow{HI} C^*H_3C(CH_3)_2 \quad | \quad I \xrightarrow[\Delta]{}$$

$$C^*H_3C{=}C^*H_2 \quad | \quad CH_3 \quad (a) \xrightarrow[Ni]{H_2} C^*H_3CH(CH_3)_2 \quad (b)$$

C. D. Wagner, D. P. Stevenson and J. W. Otvos, J. Am. Chem. Soc., *72,* 5786 (1950).

A. Procedure

(a) *2-Methylpropene-1,3-$C^{13}_{1/2}$, (Isobutylene-1,3-$C^{13}_{1/2}$).* An anhydrous solution of 2 ml. of acetone and 6 ml. of ether is added slowly to a cooled Grignard solution prepared from 2.75 g. (19.3 mmoles) of methyl-C^{13} iodide, 0.8 g. of magnesium and 12 ml. of ether. The mixture is refluxed for 10 minutes and evaporated to dryness at room temperature under reduced pressure. To a mixture of the residual 2-iodo-2-methylpropane-1-C^{13} and 1 g. of red phosphorus at $-78°$, in a flask equipped with a dropping funnel and a reflux condenser attached to a trap at $-185°$ and an Ascarite guard tube, is added slowly 12 ml. of 57% hydriodic acid. The mixture is warmed slowly and refluxed for 2 hours. The crude product is distilled at $-20°$ through an Ascarite purifying train and then separated from any ether by low-temperature distillation. The yield of product boiling over one degree is 63%.

(b) *2-Methylpropane-1-C^{13}, (Isobutane-1-C^{13}).* A stream of hydrogen at 70 ml./min. is passed successively over the 2-methylpropene-1,3-$C^{13}_{1/2}$ in a flask at $-30°$ and over a nickel-on-kieselguhr catalyst at 100°. The product is collected in quantitative yield in a trap at $-190°$.

B. Other Preparations

2-Methylbutane-1-C^{14} is prepared[1] in a similar manner from methylmagnesium-C^{14} iodide and 2-butanone, the 2-methyl-2-butanol-1-C^{14} (formed by hydrolysis of the Grignard complex) being dehydrated with sulfuric acid to form 2-methyl-2-butene-1-C^{14}.

2-Methylpropane-1-C^{14} has been prepared by Wagner and coworkers in 57% and 53% yield from 34.5 and 8 mmoles of methyl-C^{14} iodide, respectively, by a modification of this method. Ether was removed by shaking with 94% sulfuric acid at 0° and distilling the product. The use of phosphorus was eliminated in the second preparation.

The water-promoted, aluminum bromide-catalyzed, intramolecular isomerization of butane-1-C^{13} to 2-methylpropane-1-C^{13} (65%) has been described by Otvos.[2] Butane-2-C^{13} underwent no change under such conditions.

[1]J. D. Roberts, Atomic Energy Commission Report, NP-3437; Nuc. Sci. Abstracts, *5*, 7047 (1951).

[2]J. W. Otvos, D. P. Stevenson, C. D. Wagner and O. Beck, J. Chem. Phys., *16*, 745 (1948).

2-METHYLPROPANE-2-C^{13}

$$CH_3C^*OOC_2H_5 \xrightarrow{CH_3MgI} (CH_3)_3C^*OMgI \xrightarrow{HCl} \underset{(a)}{(CH_3)_3C^*Cl} \xrightarrow[\Delta]{\text{quinoline}}$$

$$\underset{(b)}{(CH_3)_2C^*{=}CH_2} \xrightarrow[Ni]{H_2} (CH_3)_3C^*H$$

C. D. Wagner, D. P. Stevenson and J. W. Otvos, J. Am. Chem. Soc., *72*, 5786 (1950).

Procedure

(a) *2-Chloro-2-methylpropane-2-C^{13}, (C_1^{13}-t-Butyl Chloride).* To a stirred Grignard solution prepared from 25.8 ml. of methyl iodide, 10.1 g. of magnesium and 60 ml. of ether is added slowly 114 mmoles of ethyl acetate-1-C^{13}, while the temperature is maintained at 25° by cooling. The mixture is stirred for 1 hour, then evaporated under reduced pressure at room temperature. The residue is cooled in an ice-bath, and 400 ml. of concentrated hydrochloric acid is added slowly through the reflux condenser. The upper layer is distilled into a 100-ml. flask, then is warmed to 0° and shaken with 100 ml. of ice-cold 65% sulfuric acid, which is added slowly to remove ether. The upper layer is distilled into two tubes, each containing 20 ml. of quinoline; yield 68 mmoles (60%).

(b) *2-Methylpropene-2-C^{13}, (Isobutylene-2-C^{13}).* The mixtures of 2-chloro-2-methylpropane-2-C^{13} and quinoline are heated at 210–230° for 2.5 hours in sealed tubes. To remove unreacted *t*-butyl chloride, the product is distilled at −78° into a bulb where it is shaken with a solution of 2 g. of silver nitrate in 10 ml. of water, then with the same solution to which is added 50 ml. of methyl cellosolve. The product then is heated for 3 hours at 100° in a sealed tube with 10 ml. of ethanolamine, to remove ethyl chloride; yield 82%.

(c) *2-Methylpropane-2-C^{13}, (Isobutane-2-C^{13}).* The 2-methylpropene-2-C^{13} is hydrogenated (96% yield) in the manner described for 2-methylpropane-1-C^{13}. The product obtained by low-temperature distillation contains less than 0.1% of nonisotopic impurities.

1-BUTENE-4-C^{14}

$$C^*H_3I \xrightarrow{Mg} \underset{(a)}{C^*H_3MgI} \xrightarrow{CH_2{=}CHCH_2Br} \underset{(b)}{CH_2{=}CHCH_2C^*H_3}$$

R. B. Regier and R. W. Blue, J. Org. Chem., *14*, 505 (1949).

A. Procedure

(a) *Methylmagnesium-C^{14} Iodide.* Magnesium ribbon (2.8 g.) and 17.7 g. of absolute ether are placed in a round-bottomed flask equipped with a magnetic stirrer, a low-temperature reflux condenser, a dropping funnel, and a side tube for introduction of dry nitrogen near the bottom of the flask. The flask is cooled and evacuated; then, with the reaction flask at 0° and the cold finger at −78°, 6.8 ml. of methyl-C^{14} iodide is added by distillation at the rate of 6.5 g. per hour. After standing overnight, the Grignard reagent is siphoned off through glass wool into a similar reaction flask, three 15-ml. portions of absolute ether being used as rinse. While a slow stream of nitrogen is passed through the system, the flask is heated slowly to 130° to remove ether (Note 1).

(b) *1-Butene-4-C^{14}.* When the evolution of ether from the methylmagnesium-C^{14} iodide becomes slow, the reaction flask is cooled to 70°, crushed ice is placed in the cold finger, and 12.7 g. of allyl bromide is added through the dropping funnel at the rate of a few drops every 30 seconds. Atmospheric pressure is maintained by slowly passing nitrogen through the system, and the product is collected in a trap at −78° and transferred by distillation into a small distilling apparatus. Using customary procedures for light-hydrocarbon distillation, the product is separated into three fractions: C_3 and lower-boiling materials, 0.1 g; C_4, 4.4 g.; C_5 and higher-boiling materials, 1.8 g. The yield is 63% and the radiochemical yield is 65.3%, both based on methanol (Note 2).

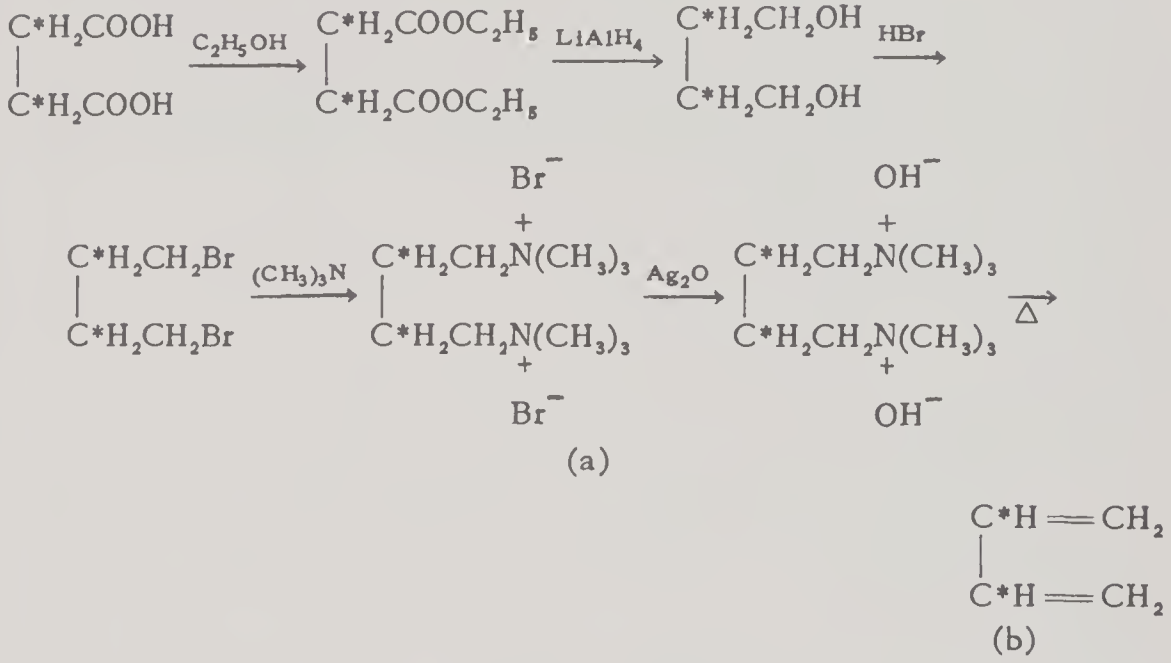
B. Notes

1. The heating schedule is: 0–40 minutes, 65–100°; 40–60 minutes, 100–120°; 60–80 minutes, 120–130°.

2. The infrared absorption spectrum of the product prepared in trial runs by this procedure was indistinguishable from that of 99.7% pure 1-butene. Impurities are less than 1% butane, 1% isobutane, 0.5% 2-butenes and 0.5% isobutene. The procedure has been employed by Wagner;[1] yield 65% based on methyl iodide.

[1]C. D. Wagner, D. P. Stevenson and J. W. Otvos, J. Am. Chem. Soc., 72, 5786 (1950).

1,3-BUTADIENE-2,3-C_2^{14}



(a)

(b)

K. M. Mann and R. F. Nystrom, J. Am. Chem. Soc., 73, 5894 (1951).

A. Procedure

(a) *Tetramethylene-2,3-C_2^{14}-bis(trimethylammonium Bromide).* The compound is prepared in four steps without isolation of intermediates. A mixture of 1.18 g. (10 mmoles) of succinic-2,3-C_2^{14} acid, 10 ml. of absolute ethanol saturated with dry hydrogen chloride (10°) and 40 ml. of benzene is refluxed for 15 hours. The water formed is removed with the use of a water trap. The excess ethanol and remaining water are removed by distillation; then the bulk of the benzene is removed at room temperature (15 mm.). The ethyl succinate-2,3-C_2^{14} is reduced by adding a solution in 25 ml. of absolute ether, dropwise, to a well-stirred solution of 1 g. of lithium aluminum hydride in 150 ml. of ether. The mixture is refluxed for 1 hour; then the excess reagent is decomposed by the dropwise addition of water. Sufficient 6 *N* hydrochloric acid is added to clear the solution, and 1,4-butanediol-2,3-C_2^{14} is removed by continuous ether extraction (74 hours). The solvent is removed in a stream of nitrogen (Note 1).

Water (10 ml.) is added to the diol, and the mixture is saturated at 10° with hydrogen bromide, then heated to 90–95° for 2 hours while hydrogen bromide is still passed in (Note 2). The 1,4-dibromobutane-2,3-C_2^{14} is extracted into five 30-ml. portions of ether. The extract is washed with

brine containing sodium bicarbonate, then dried over calcium sulfate, and the solvent is removed by evaporation at 60° under anhydrous conditions.

To the 1,4-dibromobutane-2,3-C_2^{14} is added 25 ml. of absolute ethanol which has been saturated at 10° with anhydrous trimethylamine. The mixture is refluxed for 1 hour under anhydrous conditions (Note 3); then the diquaternary salt is precipitated by dilution with 200 ml. of absolute ether and stored overnight at 4°. The salt is collected and thoroughly washed with ether; yield 2.16 g. (65%).

(b) *1,3-Butadiene-2,3-C_2^{14}*. A mixture of 1.8 g. of tetramethylene-2,3-C_2^{14}-bis(trimethylammonium bromide), 15 ml. of water, and the washed silver oxide from 2.5 g. of silver nitrate (prepared with 10 ml. of 8% potassium hydroxide) is stirred vigorously and filtered.

The substituted ammonium hydroxide is decomposed by boiling the filtered solution in a stream of helium (Note 4). Some of the water vapor in the gas stream is removed by a cold-water condenser, and the trimethylamine is absorbed by bubbling through 3 *N* sulfuric acid. The product is collected in a series of three liquid-nitrogen traps. As the reaction proceeds, the temperature is raised until, at completion, the temperature of the heating mantle is 250°. The butadiene-2,3-C_2^{14} is dried by vacuum distillation (0.1 μ) through Drierite (Note 5); yield 4.55 mmoles (84%), the vapor pressure is 9.65 cm. at −46° (Note 6).

The purity of butadiene-2,3-C_2^{14} prepared by this procedure has been established by the constant specific activities of the succinic-2,3-C_2^{14} acid, the derivative *cis*-4-cyclohexene-1,2-dicarboxylic-4,5-C_2^{14} anhydride (Note 7), and the glyoxal-C_2^{14} (Note 8) prepared from it by degradation.

B. Notes

1. Solvent removal is facilitated by use of a Cheronis porous microdisperser apparatus.

2. A cloudiness soon develops and the dibromide settles out.

3. The excess trimethylamine is allowed to escape through a mercury bubbler.

4. Glas-Col heater temperature 160°.

5. Fifteen passes through "indicating-Drierite" are required.

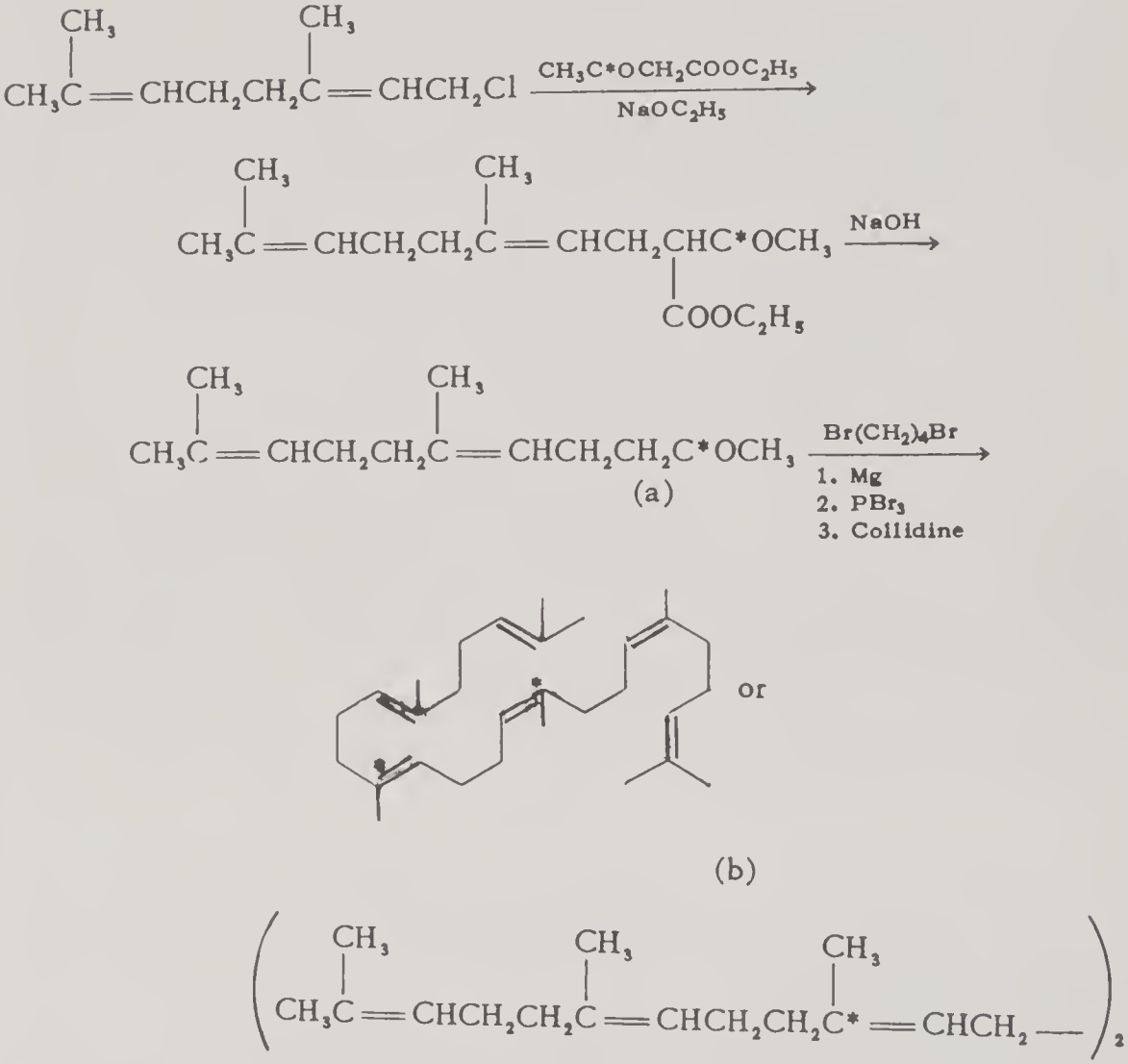
6. Butadiene of 99% purity has a vapor pressure of 9.50 cm. at −46°.

7. *cis*-4-Cyclohexene-1,2-dicarboxylic-4,5-C_2^{14} anhydride was prepared in almost quantitative yield by the reaction between butadiene-2,3-C_2^{14} and maleic anhydride.[1]

8. Glyoxal-C_2^{14} was obtained by degradation of the butadiene-2,2-C_2^{14} with ozone, and isolated as glyoxal-C_2^{14} 2,4-dinitrophenylosazone, m.p. 320–322° (dec.).

[1]E. H. Farmer and F. L. Warren, J. Chem. Soc., *1929*, 897.

2,6,10,15,19,23-HEXAMETHYL-2,6,10,14,18,22-TETRACOSAHEXAENE-10,15-C_2^{14} (C_2^{14}-Squalene)



W. G. Dauben and H. L. Bradlow, J. Am. Chem. Soc., 74, 5204 (1952).

A. Procedure

(a) *6,10-Dimethyl-5,9-undecadien-2-one-2-C^{14}* (Note 1). Ethyl acetoacetate-3-C^{14} (22.53 g.) is added dropwise to a cooled, stirred solution of 3.46 g. of sodium in 100 ml. of absolute ethanol, and the mixture is stirred for 15 minutes. Freshly distilled geranyl chloride (Note 2) is added dropwise, and the mixture is refluxed for 25 hours, then diluted with 500 ml. of water and extracted with ether. The ether is evaporated and the ethyl 2-acetyl-1-C^{14}-5,9-dimethyl-4,8-decadienoate is refluxed for 48 hours with a solution containing 350 ml. of alcohol, 230 ml. of water and 8 g. of sodium hydroxide. The mixture is diluted with two 250-ml. portions of ether. The combined extract is washed with water and

saturated sodium chloride solution, then dried over sodium sulfate and vacuum-distilled; yield 15 g. (52.5%), b.p. 115° (1 mm.) (Note 3).

(b) *2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene-10,15-C_2^{14}* (Note 4). A mixture of 15.0 g. of 6,10-dimethyl-5,9-undecadien-2-one-C_2^{14}, 10 g. of 1,4-dibromobutane, 2.3 g. of magnesium and a crystal of iodine is heated in a nitrogen atmosphere for 30 minutes on a steam-bath. Absolute ether (45 ml.) is added, and the mixture is refluxed overnight, another crystal of iodine being added at 45 minutes. The mixture is hydrolyzed with water and dilute hydrochloric acid, then the ether extract is dried and concentrated by distillation. Anhydrous benzene (30 ml.) and 15 ml. of phosphorus tribromide are added and the solution is heated for 12 hours on a steam-bath. The mixture is cooled, poured into dilute hydrochloric acid and ice-water, and the aqueous layer is separated and extracted with ether. The extracts are combined, washed with dilute alkali, water and saturated sodium chloride solution, then dried, and the solvents are distilled. The residue is refluxed for 3 hours with 60 ml. of collidine. The mixture is diluted with water and extracted with four portions of ether. The extract is washed, dried and fractionally distilled; yield 3.1 g. (19.7%), b.p. 210–212° (1.5 mm.). Paper chromatography and radioautography indicate that the product is identical with the squalene[1] regenerated from the hexachloride prepared from the naturally occurring compound (Note 5).

B. Notes

1. The procedure is a modification of the method of Carroll.[2]

2. The chloride may be prepared in 60% yield by the method of Ruzicka,[3] using phosphorus pentachloride and diluting with hexane. The thionyl chloride method appears to be less satisfactory.

3. The yield in the ketonic cleavage is consistently 90%.

4. The method is that of the Barbier reaction described by Schmitt,[4] who reported a yield of 38%, but the method is modified, for the infrared spectrum of the distilled product shows the presence of a hydroxyl group. Treatment with phosphorus tribromide and collidine yields the pure hydrocarbon. A complete chemical degradation of C^{14}-squalene is described by Cornforth.[5]

5. The paper used is impregnated with stearato chromic chloride and developed with methanol;[1] R_f 0.71 and 0.86 (weak). The infrared absorption spectra indicate that regenerated squalene possesses the structure $RR'C{=}{=}CH_2$ in addition to the structure $RR'C{=}{=}CHR''$, which is characteristic of natural squalene.

C. Other Preparations

The conversion of C^{14}-squalene (biosynthetic) to two isomeric hexachlorides, m.p. 108° and 144°, has been described by Langdon.[6]

The synthesis of a physiologically inactive isomer of 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene-10,15-C_2^{14} has been reported by Langdon and Bloch.[7] Ethyl acetoacetate-1,3-C_2^{14} was condensed with geranyl chloride in the presence of sodium ethoxide.[8] The ethyl 2-acetyl-1-C^{14}-5,9-dimethyl-4,8-decadienoate-1-C^{14}, (ethyl geranylacetoacetate-1,3-C_2^{14}), was saponified with barium hydroxide to give 6,10-dimethyl-5,9-undecadien-2-one-2-C^{14}, which was reacted[4,9] with the Grignard reagent prepared from 1,4-dibromobutane, giving 2,6,10,15,19,23-hexamethyl-2,6,18,22-tetracosatetraene-10,15-diol-10,15-C_2^{14}, (C_2^{14}-dihydroxydihydrosqualene), b.p. 165–183° (10 μ), n_D^{30} 1.4875. From the enediol was prepared[9] a crystalline hydrochloride which was identical with squalene hexahydrochloride. Dehydrohalogenation in refluxing pyridine gave the product, which was isolated by chromatography on alumina.

[1]W. G. Dauben, H. L. Bradlow, N. K. Freeman, D. Kritchevsky and M. Kirk, J. Am. Chem. Soc., *74*, 4321 (1952).

[2]M. F. Carroll, J. Chem. Soc., *1940*, 704.

[3]L. Ruzicka, Helv. Chim. Acta., *6*, 492 (1923).

[4]J. Schmitt, Ann., *547*, 115 (1951).

[5]J. W. Cornforth and A. G. Popjak, Biochem. J., *58*, 403 (1954).

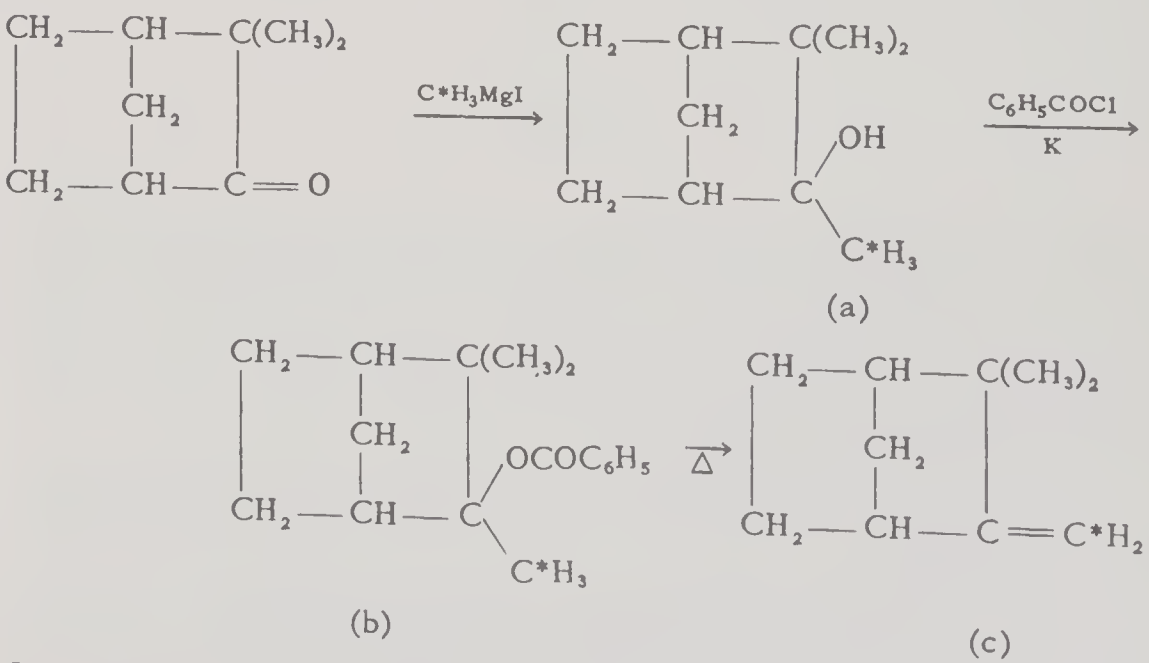
[6]R. G. Langdon and K. Bloch, J. Biol. Chem., *200*, 129 (1953); J. Am. Chem. Soc., *74*, 1869 (1952); I. M. Heilbron, E. D. Kamm and W. M. Owens, J. Chem. Soc., *1926*, 1630.

[7]R. G. Langdon and K. Bloch, J. Biol. Chem., *200*, 135 (1953).

[8]M. D. Forster and D. Cardwell, J. Chem. Soc., *103*, 1338 (1913).

[9]E. H. Farmer and D. A. Sutton, J. Chem. Soc., *1942*, 116.

CAMPHENE-8-C^{14}



J. D. Roberts and J. A. Yancey, J. Am. Chem. Soc., *75*, 3165 (1953).

A. Procedure

(a) *3,3-Dimethyl-2-methyl-C^{14}-2-norcamphanol.* To a solution of methylmagnesium-C^{14} iodide, prepared from 30.8 g. of methyl-C^{14} iodide and 6.2 g. of magnesium in 100 ml. of absolute ether, is added slowly over 2 hours a solution of 29.2 g. of camphenilone (Note 1) in 60 ml. of ether. The mixture is refluxed for 3 hours; then a solution of 30.8 g. of non-isotopic methyl iodide in 70 ml. of ether is added over a period of 30 minutes. The mixture is refluxed for 15 hours, then hydrolyzed by shaking with ice and saturated ammonium chloride solution. The aqueous layer is separated and extracted with ether, and the combined extract is dried over magnesium sulfate. The solvent is evaporated and the residue is sublimed, yielding 32.6 g. of product (99%), m.p. 113.8–115.6° (Note 2).

(b) *3,3-Dimethyl-2-methyl-C^{14}-2-norcamphanyl Benzoate.* A solution of 7.61 g. of the alcohol in 60 ml. of benzene is added rapidly to a cooled, stirred mixture of 1.93 g. of potassium-sand and 100 ml. of benzene. The mixture is refluxed until most of the metal has dissolved (2.5 hours). To the cooled mixture is added 7.0 g. of redistilled benzoyl chloride in 60 ml. of benzene over a period of 10 minutes. The mixture is stirred for 17 hours at room temperature; then 150 ml. of water is added. The benzene layer is separated, washed with sodium bicarbonate solution, then dried over magnesium sulfate and distilled (Note 3); yield 10.8 g. (84%), b.p. 118.8–122.0° (0.7–0.9 mm.).

(c) *Camphene-8-C^{14}.* The ester (1.10 g.) is heated at 300–320° in a small flask equipped with a 4-cm. Vigreux column. The yield of product boiling below 165° is 0.48 g., 83% (Note 4). Degradation studies indicate that there is 2–6% isotopic rearrangement (Note 5), probably to the methyl side chain, C-9 and C-10.

B. Notes

1. The camphenilone is prepared conveniently by the nitrogen dioxide oxidation[1] of camphene, and is purified through the semicarbazone; see Note 5. Directions are given by Vaughan[2] for preparing optically active camphenilone by resolution of 3,3-dimethyl-2-norcamphanamine, followed by conversion of the primary amine to the carbonyl function by a modification of the Sommelet reaction.[3]

2. Vaughan and Perry[2] report a melting point of 118–119° after repeated sublimation; carbanilate, m.p. 125.6–126.6°; *p*-nitrobenzoate, m.p. 143.4–144.4°.

3. The ester is fractionated through a small, wire-helix-packed column. Another preparation boiled at 113–138° (1.7 mm.), n_D^{25} 1.5318.

4. Vaughan and Perry[2] report: m.p. 49.0–52.8°, b.p. 150–154°, an infrared spectrum identical with that of an authentic sample, and a molar specific activity unchanged from that of the carbinol.

5. Camphene-8-C^{14} (1.5–2.0 g.) in 10 ml. of hexane solution at 0° is treated with tank nitrogen dioxide. The solvent is evaporated, and the crude addition product is refluxed with 30% potassium hydroxide solution for several hours. The resulting camphenilone is steam-distilled, extracted into ether and converted to the semicarbazone, which is recrystallized from dilute alcohol. This ketone contains all the carbons of the starting material except C-8.

Camphene-8-C^{14} (0.3–0.5 g.), in 10–15 ml. of chloroform solution at 0°, is treated with a slight excess of ozone. The solvent is evaporated under reduced pressure, and the residue is refluxed with 70 ml. of water for 30–45 minutes. The mixture is filtered, 300 ml. of a saturated solution of dimedon is added to the filtrate, and the mixture is set aside overnight to crystallize. The formaldehyde dimedon is collected and recrystallized from dilute methanol. This compound contains only C-8 of the starting material.

C. Other Preparations

3,3-Dimethyl-2-methyl-C^{14}-2-norcamphanol has been prepared by Vaughan and Perry[2] (86.6% yield based on camphenilone) by the procedure described; m.p. 118–119°.

Camphene-8-C^{14} has been prepared by Vaughan and Perry[2] (71.5% yield) by a similar reaction employing the xanthate ester dehydration of 3,3-dimethyl-2-methyl-C^{14}-2-norcamphanol. The ester was formed by treating the potassium alkoxide with carbon disulfide and excess methyl iodide. Roberts and Yancey[4] have prepared camphene-8,9,10-$C^{14}_{1/3}$ (59% yield) by dehydrating 3,3-dimethyl-2-methyl-C^{14}-2-norcamphanol by heating with pyruvic acid. The product was found to be 52% isotopically rearranged.

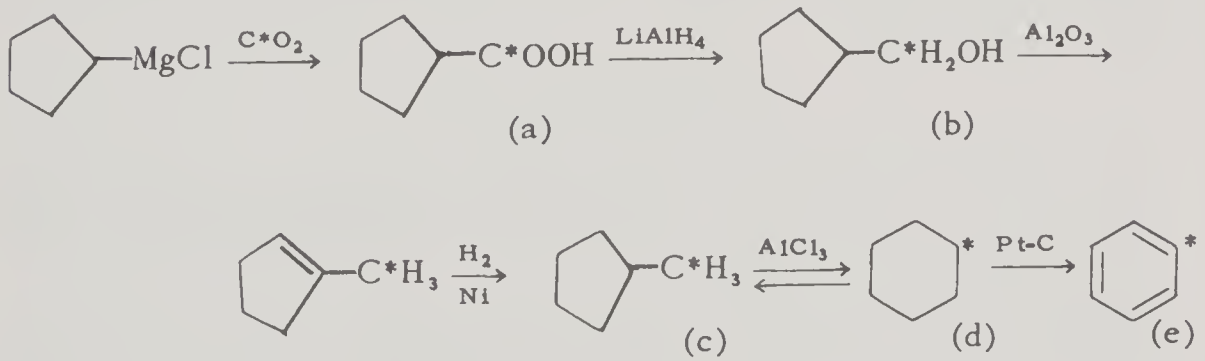
Optically active camphene-8-C^{14} has been prepared[2] from optically active camphenilone for racemization studies; m.p. 49.0–52.8°, $[\alpha]_D^{26}$ + 103.5° (benzene). The product, racemized (48% yield) by the method of Meerwein[5] through the camphene-8-C^{14} hydrochloride, m.p. 124–128°, has been degraded by ozonolysis[6] to camphenilone and the more tractable dimethylnorcampholide which had a molar specific activity that was 66.3% of that of the starting material. The interpretation has been made that camphene-8,9,10-$C^{14}_{1/3}$ is formed by a mechanism[2,4] involving both the Nametkin and Wagner-Meerwein rearrangements. Optically active camphene-8-C^{14} has been racemized[4] in 78–82% and 52% yields, by heating with hydrated titanium dioxide catalyst or with aniline hydrochloride.

The reaction is accompanied by 4–22% isotopic rearrangement (Wagner, hydrogen shift). Racemization in 35–56% yield, by heating with pyruvic acid, is accompanied by 22–61% isotopic rearrangement (Nametkin, methyl group shift).

[1]P. Lipp, Ann., *399*, 241 (1913).
[2]W. R. Vaughan and R. Perry, Jr., J. Am. Chem. Soc., *75*, 3168 (1953).
[3]J. Graymore and D. R. Davies, J. Chem. Soc., *1945*, 293; S. J. Angyal, P. J. Morris, J. R. Tetaz and J. G. Wilson, *ibid.*, *1950*, 2141.
[4]J. D. Roberts and J. A. Yancey, J. Am. Chem. Soc., *75*, 3165 (1953).
[5]H. Meerwein and F. Montfort, Ann., *435*, 207 (1923).
[6]C. Harries and B. J. Palmén, Ber., *43*, 1432 (1910).

BENZENE-C_1^{14}

METHOD I



H. S. Turner and R. J. Warne, J. Chem. Soc., *1953*, 789; H. S. Turner, Nature, *168*, 73 (1951).

A. Procedure

(a) *Cyclopentanecarboxylic-C^{14} Acid.* A Grignard reagent, prepared from 12.8 ml. (123 mmoles) of cyclopentyl chloride and 3.3 g. (136 mmoles) of magnesium in 335 ml. of anhydrous butyl ether, is carbonated for 5 minutes at −35° with the carbon-C^{14} dioxide prepared from 88.5 mmoles of barium carbonate-C^{14} (Note 1). The mixture is stirred at −20° for 45 minutes, then poured onto a mixture of 750 g. of crushed ice and 7 g. of sodium hydroxide. The butyl ether is removed by evaporation through an efficient splash trap. When the residual volume reaches 150 ml., 25 g. of silver sulfate and 75 ml. of 10 *N* sulfuric acid are added, and the mixture is steam-distilled into the flask of a continuous extractor (Note 2). The distillate is saturated with salt and continuously extracted with ether for 36 hours. The extract is dried over calcium sulfate (Note

3), and the solvent is distilled off through a 30-cm. Vigreux column. The residue is transferred into a small distillation apparatus, and the product is collected by fractional distillation through a 14-cm. Vigreux column; yield 9.48 g. (83.2 mmoles), b.p. 112–115° (19 mm.). The radiochemical yield is 95%.

(b) *Cyclopentanemethanol-α-C^{14}.* A solution of 8.52 g. (74.7 mmoles) of cyclopentanecarboxylic-C^{14} acid in 50 ml. of absolute ether is added gradually during 30 minutes to a stirred ice-cooled solution of 5.0 g. of lithium aluminum hydride (130% excess) in 250 ml. of absolute ether. After 1.5 hours of stirring at 0°, the mixture is decomposed cautiously with 10 ml. of water, followed by 100 g. of ice and 65 ml. of 10 *N* sulfuric acid. The aqueous layer is separated and extracted with five 25-ml. portions of ether. The combined extract is washed with two 10-ml. portions of saturated potassium carbonate and dried (Note 3) over anhydrous potassium carbonate and calcium sulfate. The solvent is distilled off through a column (Note 4), and the product is collected by distillation in the apparatus used above. When distillation ceases, 1.00 ml. (0.93 g.) of carrier cyclopentylmethanol is added, and the distillation is completed. The yield is 7.985 g. (79.85 mmoles), n_D^{20} 1.4572; the radiochemical yield is 95% (Note 5).

(c) *Methyl-C^{14}-cyclopentane.* Dehydration is effected by the dropwise addition of 7.81 g. (78.1 mmoles) of cyclopentanemethanol-α-C^{14}, over a period of 1 hour in a slow stream of nitrogen, to the top of a vertical column of 7 to 10 mesh granulated alumina at 320° (Note 6). The reaction products are collected in traps at −78° and −196°. After 1.25 additional hours of sweeping, the traps are attached to a vacuum manifold, and the products are distilled into a flask containing 5 g. of potassium hydroxide pellets for drying. After shaking at room temperature for a few minutes, the products are vacuum-distilled at −30° into a weighing flask; yield 6.06 g., 94.5% (Note 7).

The 1-methyl-C^{14}-cyclopentene is vacuum-distilled into the reservoir of a vaporizer unit (Note 1). The reservoir is heated in a bath at 50°, and the vapors, mixed with a stream of excess hydrogen, are carried over a bed of nickel catalyst at 150° (Note 8). The product is collected in the two traps described above. Vaporization requires 3.5 hours and the system is swept for an additional 3 hours. The product is distilled into a flask containing glass beads and 2 g. of phosphorus pentoxide for drying, and then into a weighing flask; yield 6.17 g. (94% based on the carbinol). Infrared analysis and the refractive index (Note 9) show this product to be a mixture of methyl-C^{14}-cyclopentane and cyclohexane-C_1^{14} (34%).

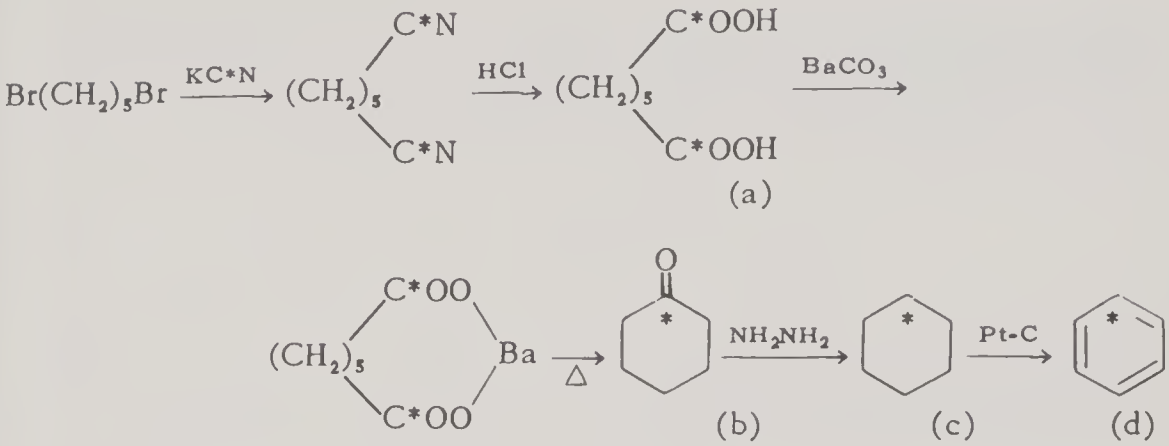
(d) *Cyclohexane-C_1^{14}.* The crude methyl-C^{14}-cyclopentane is distilled into a flask containing 10 g. of glass beads and 0.75 g. of aluminum

chloride powder. Water (0.03 ml.) is then added by vacuum distillation, and the constricted neck is sealed off. The rearrangement (Note 10) is carried out by: (1) heating the reaction mixture for 5 hours in refluxing carbon tetrachloride, (2) shaking at 30° for 2 weeks, (3) shaking at 20° for 4 weeks and (4) standing at 10–15° for 5 weeks. The mixture then is frozen in liquid nitrogen, and the flask (Note 11) is attached to a vacuum manifold by means of an adapter packed with glass wool. The product is distilled into a trap containing 2 g. of moist potassium hydroxide pellets (to destroy any catalyst), then dried over phosphorus pentoxide, as described above, and transferred to a weighing tube; yield 6.13 g. (93.5% based on the carbinol). The refractive index corresponds to 90% cyclohexane (Note 12).

(e) *Benzene-C_1^{14}*. The crude isomerization mixture (6.10 g.) is distilled into the reservoir of the vaporizer unit already described. The reservoir is heated in a bath at 60–75°, and the vapors, mixed with a slow stream of nitrogen, are carried over a bed of platinized-carbon catalyst heated to 360° (Note 13). The dehydrogenated product is collected in traps at −78° and −196°. The volatilization requires 2.5 hours, and sweeping is continued for another 4.5 hours. After drying over phosphorus pentoxide, the mixture of benzene-C_1^{14} and methyl-C^{14}-cyclopentane weighs 5.49 g., n_D^{20} 1.4925.

The crude product (5.24 g.) is sealed into a soft-glass ampoule, which is placed in a closed, heavy-walled bottle containing 450 ml. (0.25 mole) of ammoniacal nickel cyanide solution (Note 14). The ampoule is broken by shaking, which is continued for 16 hours. The mixture is cooled at 0°, and the clathrate compound is collected by filtration, washed with water, methanol and absolute ether, then dried in a vacuum desiccator; yield 12.68 g. (Note 15). A solution of 13.5 g. of potassium cyanide and 3.5 g. of potassium hydroxide in 35 ml. of water is added to 12.58 g. of the complex in a flask fitted with a gas inlet and attached, by way of a Vigreux column and dephlegmator, to cold traps (separated by a stopcock) at −78° and −196°. Most of the ammonia is removed from the distilled benzene-water by interchanging of the cooling baths. In a slow stream of nitrogen the mixture is heated in a bath at 90°, and the liberated benzene is distilled, under partial reflux, into the receivers during 3.5 hours. The total distillate is freed from permanent gases at the vacuum manifold and then distilled into a flask containing 10 ml. of syrupy phosphoric acid at −196°. After being shaken at room temperature, the wet benzene is dried (by the techniques used above) over 5 g. of powdered potassium hydroxide and finally over phosphorus pentoxide. The pure benzene-C_1^{14} weighs 4.645 g., f.p. 5.4°, n_D^{20} 1.5010 (Note 16). The chemical yield is 81% based on the carbinol; the radiochemical yield is 75% based on barium carbonate.

METHOD II



R. J. Speer, M. L. Humphries and A. Roberts, J. Am. Chem. Soc., 74, 2443 (1952).

A. Procedure

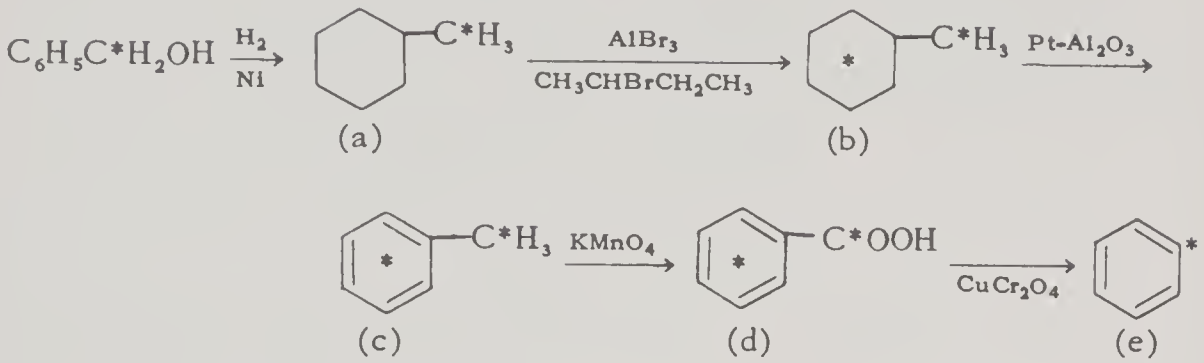
(a) *Heptanedioic-1,7-C_2^{14} Acid, (Pimelic-1,7-C_2^{14} Acid)* (Note 17). A mixture of 2.3 g. (10 mmoles) of distilled pentamethylene bromide, 1.43 g. (22 mmoles) of potassium cyanide-C^{14}, 1.5 ml. of water and 6.5 ml. of alcohol is refluxed for 3 hours. The solvent is evaporated, and the pimelonitrile-C_2^{14} is refluxed for 2 hours with 5.0 ml. of concentrated hydrochloric acid. The mixture is continuously extracted with ether for 12 hours; then the extract is dried over sodium sulfate and evaporated with a stream of dry nitrogen. The residue is dried in a vacuum desiccator at 60° for 3 hours; yield 1.465 g., 91.6% (Note 18).

(b) *Cyclohexanone-1-C^{14}*. An intimate mixture of 1.6 g. (10 mmoles) of pimelic-1,7-C_2^{14} acid and 0.05 g. (0.25 mmole) of barium carbonate is heated gradually by raising a micro furnace, preheated to 320–325°, around the flask of a distillation apparatus (Note 19) and maintaining the temperature for 4 hours. The yield of crude product is 0.8 g. (Note 20).

(c) *Cyclohexane-C_1^{14}* (Note 21). A mixture of 1.96 g. (20 mmoles) of cyclohexanone-1-C^{14}, 2.8 g. (50 mmoles) of potassium hydroxide, 20 ml. of triethylene glycol and 2.0 ml. of 85% hydrazine hydrate is refluxed for 1.5 hours. The aqueous phase is distilled until the flask temperature reaches 175–185°; then the upper layer of distillate is separated and dried over calcium chloride; yield 1.241 g., 74% (Note 22).

(d) *Benzene-C_1^{14}*. The dehydrogenation apparatus consists of an all-glass, vapor phase reactor employing a platinum-on-charcoal catalyst at 290–310° (Note 23) and a condensing system at −78°. The cyclohexane-C_1^{14} is distilled in a slow stream of hydrogen into the catalyst chamber over a period of 1 hour. On the basis of infrared analysis, the yield is 96.4% (88.5% purity), or 87.3% conversion (Note 24).

METHOD III



H. Pines and R. W. Meyerholtz, Jr., J. Am. Chem. Soc., 77, 5392 (1955).

A. Procedure (Note 25)

(a) *Methyl-C^{14}-cyclohexane.* Benzyl-α-C^{14} alcohol (18.3 g.) is hydrogenated over 3 g. of nickel-kieselguhr[1] at 110 atmospheres (initial) in a rotating autoclave. The temperature is maintained at 110–120° for 2 hours and at 140–150° until the pressure remains constant, the hydrogen uptake corresponding to the theoretical; yield 9.85 g. (65%), b.p. 97–100°, n_D^{20} 1.4230.

(b) *Methylcyclohexane-$C^{14}_{1/5}$* (Note 26). A mixture of 51 mmoles of methyl-C^{14}-cyclohexane (Note 27) and a catalyst consisting of 2.08 mmoles of anhydrous aluminum bromide (Note 28), and 1.1 mmoles of hydrogen bromide (Note 29) is introduced by vacuum technique into a reaction tube containing 0.126 mmoles of 2-bromobutane (Note 30) in a sealed ampoule. The evacuated tube (10^{-6} mm.) is sealed and the ampoule is broken. The mixture is shaken at room temperature for 21 hours, distilled under vacuum (Note 31), washed with three portions each of 10% potassium carbonate and water, then dried over calcium chloride.

(c) *Toluene-$C^{14}_{1/5}$* (Note 32). The crude methylcyclohexane-$C^{14}_{1/5}$ is passed over 18 ml. of platinized alumina[2] at 284–288° until no more hydrogen is evolved (4 times); yield 3.0 g., n_D^{20} 1.4880 (Note 33), purity 90%. Purification is accomplished by chromatography on silica gel (Note 34) and elution with absolute alcohol, the product being collected when the effluent reaches n_D^{20} 1.4752; yield 2.2 g. Degradation studies indicate that 31% of the activity is in the ring.

(d) *Benzoic-$C^{14}_{1/5}$ Acid.* A mixture of the toluene, 15 g. of potassium permanganate and 230 ml. of water is refluxed for 20 hours. The excess permanganate is destroyed with 5 ml. of methanol, and the mixture is filtered hot, with thorough washing of the manganese dioxide with 200 ml. of hot water. The solution is concentrated to 50 ml., acidified with concentrated hydrochloric acid and cooled with an ice-bath. The product

is collected, washed with ice-water and dried in a desiccator; yield 60%, m.p. 121–121.5° (cor.).

(e) *Benzene-C_1^{14}*. The benzoic acid is decarboxylated under nitrogen with the use of 0.8 g. of copper chromite catalyst and 10 ml. of redistilled quinoline according to the method of Hughes and Reid (see toluene-1,3,5-C_3^{14}); yield 75–80%, n_D^{20} 1.5011 (Note 35). The molar specific activity is 31% of that of the starting material.

B. Notes

1. Very detailed and illustrated directions are given in the later paper for all these synthetic steps. The procedure is adaptable to the 10-mmole scale.[2] For a diagram of a vaporizer unit see formaldehyde-C^{14}, Figure VI, 2.

2. The distillate amounts to 500 ml.

3. Drying is effected by refluxing over drying agent in a Soxhlet apparatus.

4. A 12-in. Dufton column is used.

5. Cyclopentanemethanol boils at 159–161°. An aliquot was converted into the 1-naphthylcarbamate, m.p. 87.5–88°, for assay. The yield on the 20-mmole scale is 96.5%.

6. The 80 × 1.3-cm. Pyrex tube is packed with 50 cm. of Spence's Type N Alumina (sulfur-free) and is preceded by a 7.5-cm. preheating section of glass beads. Before use, the catalyst is heated to 400° for 18 hours in a current of hydrogen and then swept out with nitrogen.

7. This product is a mixture of slightly moist unsaturated hydrocarbons, n_D^{20} 1.4350.

8. An 80 × 1.3-cm. Pyrex tube, with a 7.5-cm. preheating section of glass beads, is packed with 50 cm. of nickel oxide on 8–20 mesh pumice (sufficient to give a 10% nickel content on reduction). After displacement of air, the catalyst is reduced in a stream of hydrogen at 400° for 3 hours. The leads to the catalyst tube are kept at 90° by means of electrical heating tape.

In high activity runs hydrogenation over Adams catalyst at 0° in the absence of solvent has been found[4] preferable since the absorption may be followed more readily.

9. This material, n_D^{20} 1.4149, gives no yellow color with tetranitromethane in carbon tetrachloride.

10. A review of the early literature on this reaction is given by Nenitzescu and Cantuniari.[3]

The water-promoted aluminum chloride isomerization catalyst is now replaced[4] by a more efficient aluminum chloride-hydrogen chloride-alkyl halide catalyst.[5] Side reactions are reduced and the recovery of hydrocarbons is better than 97%.

11. A standard taper interjoint forms part of the flask neck below the seal.

12. Glasebrook and Lovell[6] report that the equilibrium composition, determined by refractive index, varies in favor of cyclohexane at lower temperatures. The recovery of hydrocarbon is always better than 99%, and no constituents other than methylcyclopentane and cyclohexane are in evidence from the infrared absorption spectrum. The product has n_D^{20} 1.4244.

13. Granular activated charcoal (10–16 mesh) is boiled twice with 5% nitric acid, washed, dried at 100° and heated to dull redness under vacuum. This charcoal (15.1 g.) is mixed with a solution of 9.0 g. of $H_2PtCl_6 \cdot 6H_2O$ in 50 ml. of water and evaporated to dryness with stirring. A Pyrex tube (9 mm. i.d.) is charged, to a length of 54 cm., with the catalyst which is then reduced in a current of hydrogen for 12 hours at 135° and for 4 hours at 380°. The gas is replaced by oxygen-free nitrogen.

This catalyst possesses a strong cracking activity,[4] for at 360° methylcyclohexane gives a product containing 40% benzene. A chromia-alumina catalyst (see toluene-1-C^{14}) at 500° is used for the preparation of toluene-α-C^{14}, without methyl group migration.

14. The reagent is prepared from 0.25 mole of nickel sulfate as described by Evans.[7]

15. Liquid nitrogen traps collect about 1% of the total benzene activity during the filtration.

16. A sample of 99.99% benzene melted 0.025° higher, and the refractive index was the same.

17. The procedure is adapted from that described by Cason.[8]

Loftfield[9] has modified the procedure by refluxing the halide-cyanide mixture for 2 days, then adding an excess of nonisotopic cyanide and continuing for 1 day. Hydrolysis was accomplished by refluxing with alkali for 8 hours, giving a quantitative yield of pimelic-1,7-C_2^{14} acid (m.p. 98–101°) based on the dibromide.

Pimelonitrile-C_2^{14} may be isolated by extraction into benzene, followed by fractional distillation; yield[8] 80–85%, b.p. 151–155° (3 mm.).

18. Titration shows the crude acid to be 88.4% pure, making an 81% yield of pure product.

19. The receiver flask is equipped with a gas scrubber containing potassium hydroxide solution to collect the evolved carbon-C^{14} dioxide (35%).

20. The crude product was assayed as the 2,4-dinitrophenylhydrazone;[10] yield 7.0 mmoles (70%), representing a radiochemical utilization of 35% based on the pimelic acid.

21. The procedure is an adaptation of the method of Huang-Minlon.[11]

22. The refractive index (n_D^{25} 1.4246) indicates that the purity is essentially 100%.

23. The catalyst[12] is activated in a stream of dry hydrogen for 30 minutes or longer.

24. The impurity is unreacted cyclohexane; analytical data is given by Speer.

25. These preparations are incidental to a tracer study of the isomerization of saturated hydrocarbons in the presence of aluminum bromide in a batch process.[13] The isomerization of methylcyclohexane to dimethylcyclopentanes by refluxing in the presence of water-promoted aluminum chloride, with continuous distillation of the lower boiling product, has been reported.[14]

26. The experimental procedure and a diagram of the vacuum system are described in detail by Pines.[13]

27. The hydrocarbon is stored over sodium and potassium in a tube equipped with a break seal.

28. Sublimed and vacuum-resublimed anhydrous aluminum bromide is stored in sealed, weighed capsules.

29. Hydrogen bromide, prepared by dropping bromine on tetralin,[15] is fractionated (Dry Ice-ether vapor tension 400 mm.).

30. The alkyl halide serves as a promoter (catalyst accelerator)[13,16] and chain initiator, the extent of isomerization being only 2% in its absence.

31. A small residue of yellow polymeric material remains.

32. The dehydrogenation procedure is essentially that studied by Pines,[2] in which the apparatus consisted of a graduated buret and a pump connected to a Pyrex glass reaction tube containing the catalyst bed, a water-cooled receiver-condenser, a trap at −80° and a calibrated gas-collecting bottle containing saturated brine.

33. Pure toluene, n_D^{20} 1.4961.

34. A 30-inch column of 60 to 200-mesh silica gel is used.[17]

35. The evolved carbon-C^{14} dioxide contains 69% of the initial activity.

C. Other Preparations

Cyclopentanone-1-C^{14} is prepared[18] similarly from hexanedioic-1,6-C_2^{14} acid; yield 70–80%.

Benzene-C_1^{14} has been prepared in 5.5 g. yield by Fields,[19] by decarboxylating 10 g. of benzoic-1-C^{14} acid over quinoline and copper oxide (see toluene-1,3,5-C_3^{14}). The use of copper at 500° gives poor yields.[4,20]

The preparation of C*-toluene by extension of the procedures of Method I has been reported by Kilner.[4] The equilibrium in the ring expansion[21] of ethylcyclopentane is more favorable than was the case with methylcyclopentane; also see benzene-C_1^{14}, Method III. Ethyl-1-$C_{1/1}^{13}$-2-$C_{1/1}^{14}$-

cyclopentane was isomerized in the presence of aluminum chloride (0°, 1 hour) or, preferably, in the presence of 95% sulfuric acid (25°, 5 hours), giving methyl-C^{14}-cyclohexane-$C^{13}_{1/4}$, which was dehydrogenated (Note 13) to toluene-α-C^{14}-1,2,3,4-$C^{13}_{1/4}$. Degradation studies (see benzoic-1,2,3,4-$C^{14}_{1/4}$ acid) indicate that there is no C^{14} in the ring, and the C^{13}-distribution is C-1 (70%) and C-2-6 (30%). When the reaction was carried out in the presence of aluminum chloride at 100° (8 hours or longer), however, the products were essentially randomly labeled with respect to both isotopes. The ring-chain isomerization of methyl-C^{14}-cyclohexane under these latter conditions gave methylcyclohexane-$C^{14}_{1/5}$ and toluene-$C^{14}_{1/5}$ having a random distribution (86% ring labeled). Both toluene and benzene are separated from cycloalkanes by chromatography on silica gel, the alicyclics being eluted with isopentane. The product is recovered from the adsorbent (98%) by pumping under vacuum, and the isopentane is distilled at −70 to −80°.

Ethyl-1-C^{13}-cyclopentane was prepared from cyclopentanecarboxylic-C^{13} acid by conversion to chloromethyl cyclopentyl ketone-C^{13}, by the Arndt-Eistert synthesis[22] (88%), reduction to α-methylcyclopentanemethanol-1-C^{13}, with lithium aluminum hydride (93.5%), and dehydration and hydrogenation, in turn, to 1-ethyl-1-C^{13}-cyclopentene (96%) and ethyl-1-C^{13}-cyclopentane (99%).

Ethyl-2-C^{14}-cyclopentane was prepared from ethyl-2-C^{14} iodide by the Grignard reaction, yielding 1-ethyl-2-C^{14}-cyclopentanol (72%), which was dehydrated over iodine to 1-ethyl-2-C^{14}-cyclopentene (91%) and in turn hydrogenated to ethyl-2-C^{14}-cyclopentane (99–100%). A similar series from ethyl-C^{14}_2 iodide yielded: 1-ethyl-C^{14}_2-cyclopentanol, 1-ethyl-C^{14}_2-cyclopentene and ethyl-C^{14}_2-cyclopentane. Toluene-α-C^{14}-1,2,3,4-$C^{14}_{1/4}$ is prepared[4] in high activity by the randomization of ethyl-C^{14}_2-cyclopentane and dehydrogenation of the methyl-C^{14}-cyclohexane-$C^{14}_{1/4}$.

By the procedures of Method I have been prepared[4] cyclohexanecarboxylic-C^{14} acid (96–97%), cyclohexanemethanol-α-C^{14} (96%), 1-methyl-C^{14}-cyclohexene (94%) and methyl-C^{14}-cyclohexane (98–99%, with use of PtO_2).

By the procedure of Method II, from tetramethylene bromide, have been prepared[23] hexanedinitrile-1,6-C^{14}_2 and hexanedioic-1,6-C^{14}_2 acid.

[1]V. N. Ipatieff and B. B. Corson, Ind. Eng. Chem., *26*, 1838 (1934).
[2]H. Pines, R. C. Olberg and V. N. Ipatieff, J. Am. Chem. Soc., *70*, 533 (1948).
[3]C. D. Nenitzescu and I. P. Cantuniari, Ber., *66*, 1097 (1933).
[4]A. E. H. Kilner, A. S. Turner and R. J. Warne, *Isotope Conference, 1954*, Vol. II, Academic Press, New York, 1954, p. 23.
[5]H. Pines, B. M. Abraham and V. N. Ipatieff, J. Am. Chem. Soc., *70*, 1742 (1948).
[6]A. L. Glasebrook and W. G. Lovell, J. Am. Chem. Soc., *61*, 1717 (1939).
[7]R. F. Evans, O. Ormrod, B. B. Goalby and L. A. K. Staneley, J. Chem. Soc., *1950*, 3346.
[8]J. Cason, L. Wallcave and C. N. Whiteside, J. Org. Chem., *14*, 37 (1949).

[9]R. B. Loftfield, J. Am. Chem. Soc., *73*, 4707 (1951).

[10]H. A. Iddles, A. W. Low, B. D. Rosen and R. T. Hart, Ind. Eng. Chem., Anal. Ed., *11*, 102 (1939).

[11]Huang-Minlon, J. Am. Chem. Soc., *68*, 2487 (1946).

[12]R. P. Linstead and S. L. S. Thomas, J. Chem. Soc., *1940*, 1127.

[13]H. Pines, B. M. Abraham and V. N. Ipatieff, J. Am. Chem. Soc., *70*, 1742 (1948).

[14]R. Van Volkenburgh, K. W. Greenlee and C. E. Boord, Abstracts of Papers, 118th Meeting of the Am. Chem. Soc., Chicago, Sept. 3, 1950, p. 36-N.

[15]J. Houben, *Die Methoden der Organischen Chemie*, 3rd ed., Vol. III, Verlag Georg Thieme, Leipzig, 1930, p. 1156; H. Pines and R. C. Wackher, J. Am. Chem. Soc., *68*, 595 (1946); *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 338.

[16]H. Pines, F. J. Pavlik and V. N. Ipatieff, J. Am. Chem. Soc., *73*, 5738 (1951); *74*, 5544 (1952).

[17]B. J. Mair and A. F. Forziati, J. Research Natl. Bur. Standards, *32*, 151, 165 (1944).

[18]M. Fields, M. A. Leaffer, S. Rothchild and J. Rohan, J. Am. Chem. Soc., *74*, 5498 (1952); M. Fields, M. A. Leaffer and J. Rohan, Science, *109*, 35 (1949).

[19]R. J. Speer, D. R. Carmody, M. L. Humphries and A. Roberts, Nucleonics, *7* (4), 46 (1950).

[20]M. L. Bender, J. Am. Chem. Soc., *73*, 1626 (1951).

[21]H. Pines and V. N. Ipatieff, J. Am. Chem. Soc., *61*, 1076 (1939); H. Pines, F. J. Pavlik and V. N. Ipatieff, J. Am. Chem. Soc., *73*, 5738 (1951); M. B. Turova-Pollak and Z. Makaeva, J. Gen. Chem. (U.S.S.R.), *9*, 1279 (1939); Chem. Abstracts, *34*, 727 (1940); O. Beek, J. W. Otvos, D. P. Stepenson and C. D. Wagner, J. Chem. Phys., *16*, 256, 745 (1948); E. F. G. Herington and E. K. Rideal. Proc. Roy. Soc., *A184*, 447 (1945).

[22]*Organic Reactions*, Vol. I, Wiley, New York, 1942, p. 38.

[23]R. J. Speer, M. L. Humphries and A. Roberts, J. Am. Chem. Soc., *74*, 2443 (1952).

TOLUENE-α-C^{14}

$$C_6H_5C^*OOH \xrightarrow{H} C_6H_5C^*H_2OH \xrightarrow[Pd\text{-}Pt]{H_2} C_6H_5C^*H_3$$

(a) (b)

C. W. Bills and A. R. Ronzio, Atomic Energy Commission Reports, AECU-1005, 1007; Nuc. Sci. Abstracts, *5*, 585, 382 (1951).

A. Procedure

(a) *Benzyl-α-C^{14} Alcohol* (Note 1). An electrolytic cell is prepared by melting a layer of pure lead (Note 2) about 3 mm. deep in the bottom of a 5 × 7-cm. beaker and extending one glass-covered lead electrode above the rim. The cell contains a glass-covered magnetic stirring bar, and suspended within the cell is a 2.5 × 8-cm. porous clay cup containing the other lead electrode. To a catholyte consisting of 7.5 ml. of water and

9 ml. of sulfuric acid in the cell is added a solution of 0.7735 g. of benzoic-C^{14} acid in 15 ml. of methanol. Sufficient 30% sulfuric acid (Note 3) is added to the porous cup to place the level of the anolyte above that of the benzoic acid solution. The reduction is carried out at a current density of 0.10 amperes per square centimeter at 30–32° with stirring and water-bath cooling (Note 4). The catholyte mixture is made basic and extracted continuously with ether for 8 hours. The extract is frozen with liquid nitrogen, and the ether and methanol are distilled off at −20° at 3–5 μ. into a trap cooled with liquid nitrogen. The yield is about 80% (Note 5).

(b) *Toluene-α-C^{14}* (Note 6). A flask containing 0.5478 g. (5.07 mmoles) of benzyl-α-C^{14} alcohol, 0.025 g. of ferrous sulfate, 0.055 g. of Adams platinum catalyst,[1] 2 drops of 10% palladium chloride solution, 5 ml. of methanol, and a glass-enclosed magnetic stirring bar is attached to a vacuum manifold. The mixture is frozen with liquid nitrogen, and the system is evacuated to 3–5 μ, then filled with hydrogen at atmospheric pressure. The carbinol is hydrogenated for 2 hours at room temperature with stirring. Upon completion of the reaction, the mixture is cooled and thoroughly mixed (15 minutes) with 3 g. of Drierite. The mixture again is frozen with liquid nitrogen, and the product is vacuum-distilled at room temperature into a liquid nitrogen-cooled trap, through a drying train containing calcium bromide (Note 7) and phosphorus pentoxide-on-glass wool. The distillation is completed by immersing the flask in warm water; yield 0.4545 g. (97.2%).

B. Notes

1. The procedure is adapted from the method of Swann and Lucker.[2] For other preparative procedures see phenylacetic-2-C^{14} acid.

2. Lead of 99.9% purity is satisfactory for the cathode, although 99.99+ per cent lead extruded cathodes may be preferable. A diagram of the cell is presented by Bills; also see Coleman and Johnson.[3] Extruded lead cathodes are superior to those of cast lead.[2]

3. Optimum yields are obtained with 30% acid.

4. Occasional additions of 30% sulfuric acid are necessary to keep the current constant. The temperature is measured by a thermometer suspended in the catholyte. Swann[2] found that the best yield was obtained with a reaction time of 9–10 hours at the 20-g. level.

5. The yield is based on the average obtained in trial runs.

6. The procedure is a modification of the method of Baltzly,[4] which employed palladized charcoal catalyst. Use of the latter catalyst for the purpose has been reported by Wheland.[5]

7. The anhydrous calcium bromide serves to remove carbinols.

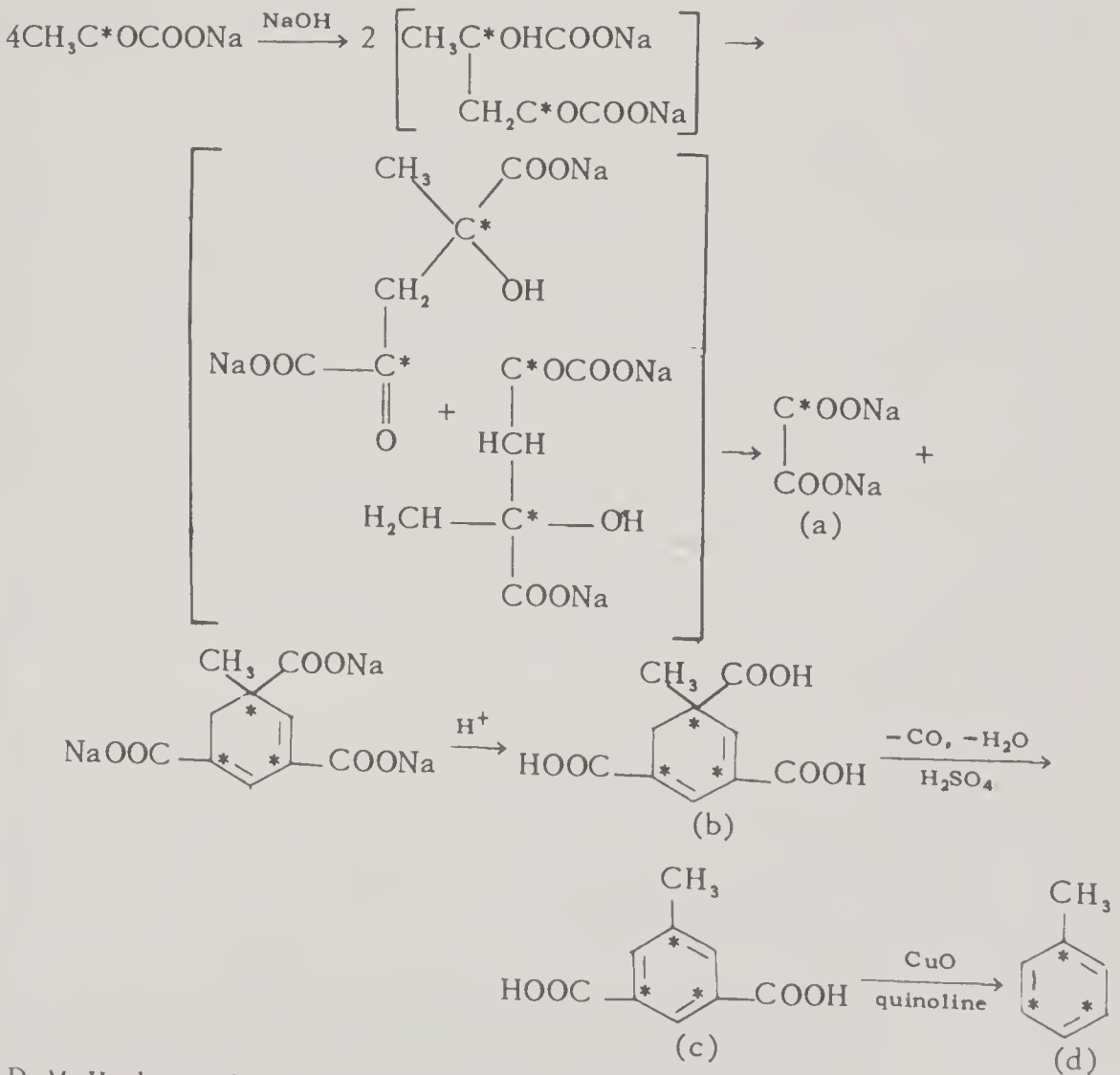
C. Other Preparations

The preparation of toluene-α-C^{14} in 73% yield by hydrogenation of ethyl benzoate-C^{14} has been reported by Heidelberger.[6]

Methyl-C^{14}-cyclohexane has been prepared,[7] in 75% yield based on barium carbonate, by hydrogenation of ethyl benzoate-C^{14} over copper chromite at 200 atmospheres for 4 hours.

[1]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 463.
[2]S. Swann, Jr., and G. D. Lucker, Trans. Electrochem. Soc., *75*, 411 (1939).
[3]*Organic Syntheses*, Vol. 21, Wiley, New York, 1941, p. 10.
[4]R. Baltzly and J. S. Buck, J. Am. Chem. Soc., *65*, 1984 (1943).
[5]G. W. Wheland and J. R. Schwartz, J. Chem. Phys., *17*, 425 (1949).
[6]C. Heidelberger, J. Biol. Chem., *179*, 139 (1949).
[7]B. A. Fries, Nucleonics, *7* (4), 47 (1950).

TOLUENE-1,3,5-C_3^{14}



D. M. Hughes and J. C. Reid, J. Org. Chem., *14*, 516 (1949).

A. Procedure

(a) *Oxalic-C_1^{14} Acid* (Note 1). To a stirred solution of 22 g. (550 mmoles) of sodium hydroxide in 39 ml. of water, cooled with an ice-bath, is added 10.604 g. (121 mmoles) of pyruvic-2-C^{14} acid over a period of 30 minutes (Note 2). The solution is heated on a steam-bath for 3.5 hours (Note 3), then chilled, and the sodium oxalate-C_1^{14} is collected on a filter and washed with three 5-ml. portions of 12 *N* sodium hydroxide; yield 84% (Note 4). Purification is accomplished by conversion to barium oxalate-C_1^{14}, then dissolution of the salt in 3 *N* hydrochloric acid and evaporation of the solution to dryness, followed by vacuum sublimation of the acid and recrystallization from water.

(b) *1,2-Dihydro-1-methyltrimesic-1,3,5-C_3^{14} Acid* (Note 1). The filtrate left after removal of sodium oxalate-C_1^{14} is cooled with an ice-bath and slowly acidified with 50 ml. of hydrochloric acid. The precipitate is collected, washed with 3-ml. portions of water (total 25 ml.) to remove sodium chloride (Note 5), and dried in vacuum at room temperature; yield 4.97 g., 67.5% (Note 6). The product is recrystallized from dilute alcohol (Note 7).

(c) *Uvitic-1,3,5-C_3^{14} Acid*, (*5-Methylisophthalic-1,3,5-C_3^{14} Acid*) (Note 1). To 4.74 g. of dry, crude 1,2-dihydro-1-methyltrimesic-1,3,5-C_3^{14} acid under nitrogen is added 20 ml. of concentrated sulfuric acid, and the mixture is heated to 120°. When the evolution of carbon monoxide ceases (Note 8), a stopcock is opened to admit a stream of nitrogen (25 ml./min.), and the temperature is raised to 150° for 2 hours. The mixture is cooled somewhat and poured into 100 ml. of water. The product is crystallized by cooling the hot solution (Note 9), and is collected, washed free of sulfate (Note 10) and dried under vacuum; crude yield 3.53 g. (100%). Purification is accomplished by treatment with carbon and recrystallization from dilute alcohol (Note 11).

(d) *Toluene-1,3,5-C_3^{14}*. The apparatus consists of a tube (heated with a salt-bath) fitted with a nitrogen inlet and connected, in turn, to a trap at room temperature, and a spiral trap cooled with Dry Ice-isopropanol and terminating in a carbon dioxide absorber (Note 12). A mixture of 3.44 g. of dry, crude uvitic-1,3,5-C_3^{14} acid, 0.50 g. of powdered cupric oxide and 10 ml. of redistilled quinoline is heated, under nitrogen, from 150° to 265° over a period of 1 hour. While the bath is held at 265 ± 5° for 3 hours, a stream of nitrogen is passed through the system. Toward the end of the reaction, toluene is distilled from the foretrap by gentle warming with a soft flame (Note 13). The distillate is frozen with liquid nitrogen, then the trap is attached to a vacuum manifold and pumped down to 5 μ. The product is distilled into a flask containing 10 g. of phosphorus pentoxide, dried and distilled into a storage receiver

(Note 12); yield 1.610 g., 91.5% based on uvitic acid, or 62% based on pyruvic acid (Note 14).

B. Notes

1. The method is that described by Wolff.[1] Two molecules of the intermediate aldol-type condensation product, "*p*-pyruvic acid," condense with elimination of water and oxalic acid.

2. The yield is lowered if the order of mixing is reversed, or if local heating is excessive.

3. The solution darkens in color and a precipitate of sodium oxalate forms.

4. The yield is based on the permanganate titration of an aliquot.

5. Continuous ether extraction of the filtrate for 8 hours yields 1.60 g. of unknown material which may be burned for isotope recovery.

6. This anhydrous acid absorbs atmospheric moisture, forming a monohydrate.

7. The temperature is kept below 60° as a precaution against decomposition.

8. Analysis of the exhaust gases shows that the carboxyl carbon is lost as carbon monoxide (96.8%); the sulfuric acid not functioning as an oxidizing agent as was postulated by Wolff.[1]

9. A product formed in the cold is difficult to filter.

10. The presence of sulfate in this intermediate leads to contamination of the derived toluene with sulfur dioxide.

11. The neutralization equivalent of a twice crystallized sample is 90.5 (calc. 90.1).

12. A diagram of the system is presented by Hughes.

13. The yield of nonisotopic barium carbonate, precipitated from the absorber, is 7.45 g. (98.5%) after correction for blank.

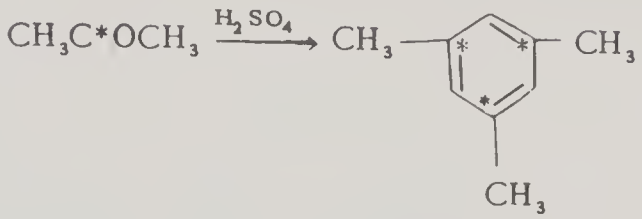
14. The product from a trial run boiled at 110.6–111.1°, n_D^{22} 1.4978. Mass spectrometric analysis indicates that the purity is 99.8% or better.

C. Other Preparations

Schepartz[2] has reported the preparation of 1,2-dihydro-1-methyltrimesic-1,3,5-C_3^{14} acid (55% yield) and uvitic-1,3,5-C_3^{14} acid (91% yield) by this method. Toluene-1,3,5-C_3^{14} was prepared[2] (70–100% yield) by fusing the uvitic acid with soda lime.[3]

[1] L. Wolff, Ann., *305*, 125 (1899).
[2] B. Schepartz and S. Gurin, J. Biol. Chem., *180*, 663 (1949).
[3] A. von Baeyer, Z. Chem., *4*, 119 (1868).

MESITYLENE-1,3,5-C_3^{14}



A. V. Grosse and S. Weinhouse, Science, *104*, 402 (1946).

Procedure

2-Propanone-2-C^{14} (1.632 g.) is condensed with sulfuric acid according to the procedure of Adams and Hufferd.[1] The crude distillate (0.296 g.) is diluted with 0.865 g. of carrier mesitylene before purification, through formation of the monosulfonic acid derivative (m.p. 74–75°) and hydrolysis back to the hydrocarbon. The product is further purified by vacuum distillation over sodium; yield 0.672 g., n_D^{20} 1.4976. The radiochemical yield is 10%. The refractive index of an authentic specimen is n_D^{20} 1.4967.

1,3,5-Trimethylcyclohexane-1,3,5-C_3^{14} is prepared by high-pressure catalytic hydrogenation of the product.

[1]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 341.

ETHYL-2-C^{14}-BENZENE

METHOD I

$$C_6H_5COC^*H_3 \xrightarrow[\substack{Pd\\HClO_4}]{H_2} C_6H_5CH_2C^*H_3$$

R. M. Roberts, G. A. Ropp and O. K. Neville, J. Am. Chem. Soc., 77, 1764 (1955); G. Ropp and O. K. Neville, Nucleonics, 7 (4), 46 (1950).

A. Procedure (Note 1)

A mixture of 2.1 ml. of acetophenone-2-C^{14}, 6 ml. of glacial acetic acid, 5 drops of 70% perchloric acid and 2 g. of 5% palladium-charcoal catalyst is hydrogenated for 72 hours at 50 p.s.i. The mixture is diluted with 100 ml. of carrier ethylbenzene, filtered and distilled; radiochemical yield 82.5%, b.p. 132° (743 mm.). Degradation studies (Note 2) indicate that there is no isotopic rearrangement.

METHOD II

$$C^*H_3CH_2Cl \xrightarrow[AlCl_3]{C_6H_6} C_6H_5CH_2C^*H_3$$

R. M. Roberts, G. A. Ropp and O. K. Neville, J. Am. Chem. Soc., 77, 1764 (1955); G. Ropp and O. K. Neville, Nucleonics, 7 (4), 46 (1950).

A. Procedure (Notes 1 and 3)

To a stirred mixture of 1.5 g. of anhydrous aluminum chloride and 21 g. of benzene is added a cold solution of 10 g. of ethyl-2-C^{14} chloride in 27 g. of benzene. The mixture is refluxed for 75 minutes and hydrolyzed with cold water. The benzene layer is washed, dried and fractionated through a 100-cm. Vigreux column; yield 4.1 g. (25%), b.p. 133–135°. Degradation studies (Note 2) indicate that there is no isotopic rearrangement (Note 4).

B. Notes

1. These preparations are incidental to a tracer study of the rearrangement of alkyl groups during Friedel-Crafts disproportionations.[1] Also see ethyl-$C^{14}_{1/2}$ chloride. Five possible reactions undergone by alkylaromatic compounds in the presence of isomerization catalysts are outlined by Roberts.

2. Directions are given by Roberts for oxidizing the ethylbenzene with concentrated nitric acid for 40 hours at 100°, forming *p*-nitrobenzoic acid; yield 10–15%, m.p. 235–236°.

Directions are given, also, for degrading the ethylbenzene to benzoic acid; yield 50%, m.p. 120.5–122°. The side chain is brominated, hydrolyzed, and oxidized with alkaline permanganate.

Experiment shows that ethyl-2-C^{14}-benzene is not rearranged by heating with anhydrous aluminum chloride in nitrobenzene solution[2] for 6 hours at 72°. The addition of small amounts of (1-chloroethyl)benzene[3] or water[4] has no influence; see C^{14}_1-*t*-amyl bromide and 2-chloro-2-methyl-propane-2-C^{14}.

When ethyl-2-C^{14}-benzene is refluxed with anhydrous aluminum chloride for 2.5 hours, a mixture of benzene, ethyl-2-C^{14}-benzene (b.p. 133–135°), and *m*- and *p*-bis (ethyl-2-C^{14})benzene (b.p. 175–185°) is formed. Degradation to benzoic, isophthalic and terephthalic acids indicates the absence of isotopic rearrangement during the intermolecular rearrangement, thereby excluding the possibility of ethylene or another symmetrical intermediate as the migrating entity.

3. The procedure is an adaptation of that of Shreiner.[5]

4. The inhibition of isomerization by aromatic hydrocarbons has been reported by Pines[6] and Roberts.[7]

C. Other Preparations

Ethyl-$C^{14}_{1/2}$-benzene has been prepared in the same manner from ethyl-$C^{14}_{1/2}$ chloride. Degradation formed benzoic-C^{14} acid having 41.8% of the molar specific activity of the starting material.

Propyl-2-C^{14}-benzene, prepared[8] by reduction of propiophenone-2-C^{14} with zinc amalgam and hydrochloric acid, has been isomerized by heating with aluminum chloride at 100° for 6.5 hours. Oxidative degradation studies disclosed that the benzoic-C^{14} acid and the mixed C^{14}-isophthalic and C^{14}-terephthalic acids contained 15.6% of the initial radioactivity. The extent of rearrangement (31.2%) is somewhat dependent upon the proportion of catalyst used.

The preparation of ethylbenzene-$C^{14}_{1/4}$ by a Friedel-Crafts reaction between gaseous benzene-C^{14} and ethyl chloride on a surface of sublimed aluminum chloride at 25° in a static system has been described by Blau;[9] yield 12.2% and 88.0% of higher ethylated benzenes on the basis of isotope dilution and infrared absorption analysis.

[1]R. E. Kinney and L. A. Hamilton, J. Am. Chem. Soc., *76*, 786 (1954); C. C. Price, *Organic Reactions*, Vol. III, Wiley, New York, 1946, p. 1; L. I. Smith, *Organic Reactions*, Vol. I, Wiley, New York, 1942, p. 370; D. Nitingale, Chem. Revs., *25*, 347 (1939); A. W. Francis, Chem. Revs., *43*, 257 (1948); C. A. Thomas, *Anhydrous Aluminum Chloride in Organic Chemistry*, Reinhold, New York, 1941; G. Egloff, *Reactions of Pure Hydrocarbons*, Reinhold, New York, 1937; D. V. Nitingale and J. M. Shakelford, J. Am. Chem. Soc., *76*, 5767 (1954).

[2]C. A. Thomas, *Anhydrous Aluminum Chloride in Organic Chemistry*, Reinhold, New York, 1941, p. 873.

[3]H. Pines, B. M. Abraham and V. N. Ipatieff, J. Am. Chem. Soc., *70*, 1742 (1948).

[4]R. C. Wackher and H. Pines, J. Am. Chem. Soc., *68*, 1642 (1946).

[5]E. Shreiner, J. prakt. Chem., (2) *81*, 557 (1910).

[6]H. Pines, E. Aristoff and V. N. Ipatieff, J. Am. Chem. Soc., *71*, 749 (1949).

[7]J. D. Roberts, R. E. McMahon and J. S. Hine, J. Am. Chem. Soc., *72*, 4237 (1950), footnote 20.

[8]R. M. Roberts and S. G. Brandenberger, Chemistry and Industry, *1955*, 227; R. Heise and A. Töhl, Ann., *270*, 155 (1892).

[9]M. Blau and J. E. Willard, J. Am. Chem. Soc., *75*, 3330 (1953).

STYRENE-α-C^{14}

$$C_6H_5C^*OCH_3 \xrightarrow[\text{cat.}]{H_2} C_6H_5\underset{\displaystyle OH}{\underset{|}{C^*}}HCH_3 \xrightarrow[\Delta]{KHSO_4} C_6H_5C^*H{=}CH_2$$

(a) (b)

G. A. Ropp, V. F. Raaen and A. J. Weinberger, J. Am. Chem. Soc., *75*, 3694 (1953).

A. Procedure

(a) *α-Methylbenzyl-α-C^{14} Alcohol.* Acetophenone-1-C^{14} is catalytically reduced—no details are given.

According to the procedure of Young,[1] acetophenone is selectively hydrogenated, in nearly quantitative yield, over 2% (by weight) of an activated Cu-Cr catalyst at 130° and 75 p.s.i. with agitation. An improved Fe-Cu-Cr catalyst for this purpose is described.[2]

(b) *Styrene-α-C^{14}.* α-Methylbenzyl-α-C^{14} alcohol is dehydrated by heating with a small amount of fused potassium bisulfate at a suitable pressure, according to the method of Brooks,[3] which follows (Note 1).

A mixture of 200 g. of *o*-chloro-α-methylbenzyl alcohol, 2 g. of powdered, fused potassium bisulfate and 2 g. of hydroquinone (to prevent polymerization) is heated at 200–210° in a 500-ml. modified Claisen flask at a pressure of 110–130 mm. The *o*-chlorostyrene, water and alcohol are distilled at 125–140° in about 1.5–2 hours. The distillate is extracted with ether, and the extract is washed with 5% sodium hydroxide solution and saturated calcium chloride solution, then dried over calcium chloride, concentrated, and vacuum-distilled. The yield is 81 g. (70%) of *o*-chlorostyrene, b.p. 62–64° (4 mm.); 60 g. of unreacted carbinol is recovered.

B. Notes

1. No isotope effect is found in forming [1,2-dibromoethyl-1(or 2)-C^{14}]-benzene by brominating the double bond in chloroform solution at –70°. The solvent is removed at reduced pressure, below 30°, and the residue is recrystallized from alcohol. There is no exchange of bromine between labeled and nonisotopic styrene.

C. Other Preparations

Styrene-β-C^{14} is prepared by the same procedure from α-methyl-C^{14}-benzyl alcohol, prepared in turn from acetophenone-2-C^{14}.

Styrene-α-C^{14} has been prepared in a similar manner by Neville.[4] Acetophenone-1-C^{14} was reduced with lithium aluminum hydride, the α-methylbenzyl-α-C^{14} alcohol was dehydrated over potassium bisulfate at 210°, and the product was purified by fractional distillation and chromatography. The thermal polymerization of styrene-α-C^{14} has been carried out successfully in a Pyrex bomb tube.

Styrene-β-C^{14} has been prepared[6] by the decarboxylation[5] of cinnamic-α-C^{14} acid (yield 50%), and used in studying the synthesis of branched polymers.

[1]D. M. Young, F. G. Young, Jr., and H. R. Guest, U.S. 2,575,403; through Chem. Abstracts, *46*, 5084 (1952).

[2]D. M. Young, F. G. Young, Jr., and H. R. Guest, U.S. 2,575,404; through Chem. Abstracts, *46*, 5085 (1952).

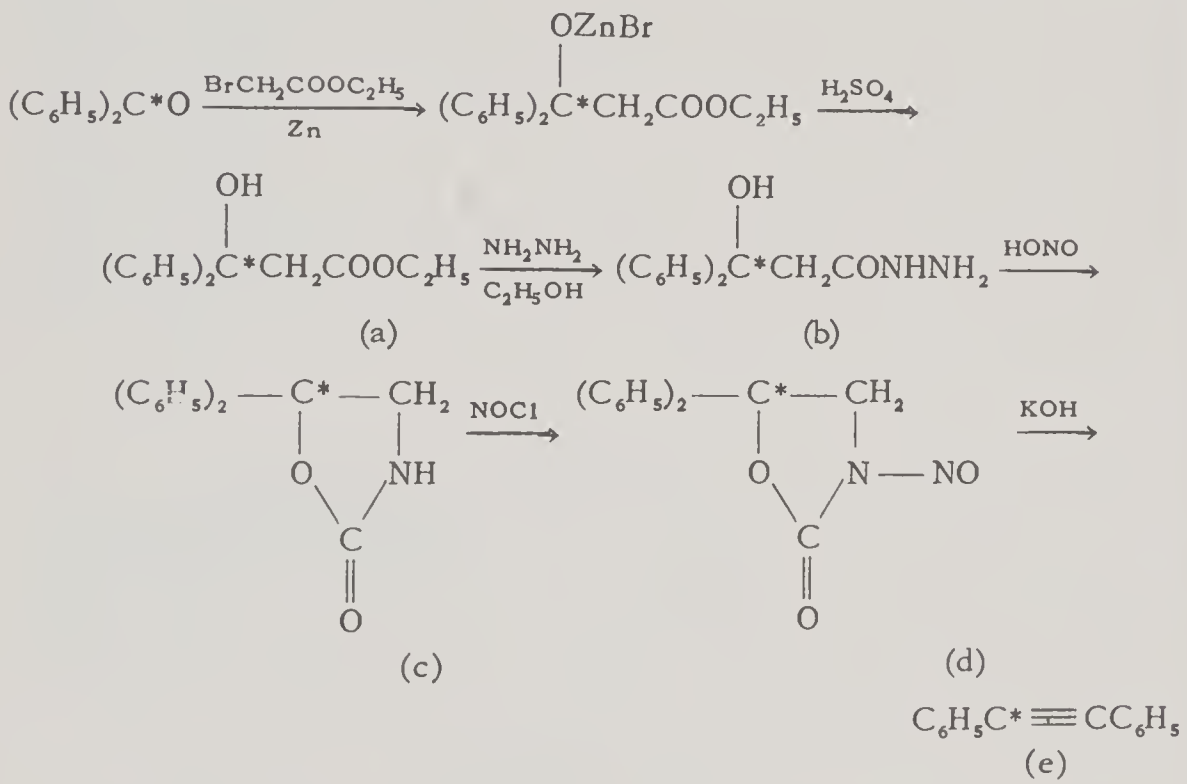
[3]L. A. Brooks, J. Am. Chem. Soc., *66*, 1295 (1944).

[4]O. K. Neville, H. E. Heller and J. Weinberger, Nucleonics, 7 (4), 48 (1950).

[5]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 440.

[6]M. H. Jones, H. W. Melville and W. G. P. Robertson, Nature, *174*, 78 (1954); J. C. Bevington, G. M. Guzman and H. W. Melville, Proc. Roy. Soc. (London), *221A*, 453 (1954).

DIPHENYLACETYLENE-C_1^{14}
(C_1^{14}-Tolan)



J. G. Burr, Jr., J. Am. Chem. Soc., *75*, 1990 (1953).

A. Procedure

(a) *Ethyl 3,3-Diphenylhydracrylate-3-C^{14}*, (*Ethyl 3-Hydroxy-3,3-diphenylpropionate-3-C^{14}*). Benzophenone-C^{14} is condensed with ethyl bromoacetate according to the procedure of Rupe,[1] which follows; yield nearly quantitative, m.p. 85–86° after recrystallization.

To a solution of 18.2 g. of benzophenone, 20 g. of ethyl bromoacetate and 90 ml. of sodium-dried benzene is added 8 g. of zinc (Note 1). The mixture is warmed under reflux until a vigorous reaction commences; then smooth boiling is maintained by suitable immersion in ice-water. Finally, after 0.75 hour of boiling, the mixture is ice-cooled and acidified with

dilute sulfuric acid. The benzene layer is separated, washed with dilute acid and water, and evaporated to dryness. The ester is crystallized from dilute alcohol as prisms, m.p. 87°; yield about 95%.

(b) *3,3-Diphenylhydracrylic-3-C^{14} Acid Hydrazide.* Ethyl 3-hydroxy-3,3-diphenylpropionate-3-C^{14} (13.5 g.) is refluxed for 3 hours with a mixture of 10 ml. of 95% hydrazine hydrate and 25 ml. of ethanol. The product is collected, washed and dried under vacuum over sulfuric acid. The yield is 16 g. of colorless solid, which melts at 130° after recrystallization from benzene.

(c) *5,5-Diphenyl-2-oxazolidinone-5-C^{14}.* A stirred solution of 7.2 g. of the acid hydrazide, 60 ml. of acetic acid and 6 ml. of 6 *N* hydrochloric acid is treated with a solution of 2.1 g. of sodium nitrite in 15 ml. of water. The suspension of solid which forms is heated on a steam-bath, under conditions of the Curtius rearrangement, until nitrogen evolution ceases (Note 2). The clear yellow solution is cooled and diluted with water to cause crystallization; yield 4.1 g., m.p. 196–198° (Note 3).

(d) *3-Nitroso-5,5-diphenyl-2-oxazolidinone-5-C^{14}.* The 5,5-diphenyl-2-oxazolidinone-5-C^{14} is nitrosated by the procedure of Newman and Kutner,[2] which follows. The product is not isolated for the next step (Note 4).

To a stirred mixture of 29 g. of 5,5-diphenyl-2-oxazolidinone and 200 ml. of anhydrous pyridine at 10–15° is added slowly, during 20 minutes, 28 ml. of 4.8 *N* nitrosyl chloride in acetic anhydride. After 5 minutes of stirring, the mixture is poured onto 250 g. of crushed ice. The yellow crystalline product is collected, washed with ice water and dried under vacuum over sulfuric acid; yield 92%, m.p. 107.5–108.5° after recrystallization from petroleum ether (b.p. 95–100°).

(e) *Diphenylacetylene-C_1^{14}.* The mixture of the nitrosoöxazolidinone and ice water is decomposed by the addition of a small excess of 50% potassium hydroxide solution, until the gas evolution is complete. The cream-colored solid is collected in 60% yield, m.p. 52–55° (Note 5). Recrystallization from dilute alcohol gives colorless needles, m.p. 56–57°.

B. Notes

1. Activated zinc may be prepared[2] by washing zinc with 5% hydrochloric acid, water and acetone, and drying.

2. The hydrazide is converted to the 3-hydroxy acid azide which rearranges to the 2-oxazolidinone,[2,3] usually around 50°.

3. Recrystallization from ethanol gives colorless crystals melting at 200°. Further methods for preparing 2-oxazolidinones are described by Newman and Kutner.[2]

4. For synthetic purposes it is not necessary to isolate these compounds, as the alkaline treatment may be carried out at once. Certain

3-nitroso-2-oxazolidinones are dangerously unstable. An alternate preparation is described[2] employing sodium nitrite.

5. The decomposition of the nitrosoöxazolidinone is about quantitative. Diphenylacetylene picrate melts at 108.5–110°.

[1]H. Rupe and E. Busolt, Ber., *40*, 4537 (1907).
[2]M. S. Newman and A. Kutner, J. Am. Chem. Soc., 73, 4199 (1951).
[3]W. J. Close, J. Am. Chem. Soc., 73, 95 (1951).

TRIPHENYLETHYLENE-$C^{14}_{1/2}$

$$C_6H_5CH_2C^*OC_6H_5 \xrightarrow{C_6H_5MgBr} C_6H_5CH_2C^*(OH)(C_6H_5)_2 \xrightarrow{H_2SO_4 \text{—} H_2O}$$

(a)

$$C_6H_5CH{=}C^*(C_6H_5)_2 \xrightarrow[\text{xylene}]{P_2O_5} C_6H_5C^*H{=}C^*(C_6H_5)_2 \xrightarrow{KMnO_4}$$

(b) (c)

$$C_6H_5C^*OOH + (C_6H_5)_2C^*O$$

C. J. Collins and W. A. Bonner, J. Am. Chem. Soc., 75, 5379 (1953).

A. Procedure

(a) *1,1,2-Triphenylethanol-1-C^{14}* (Note 1). To a Grignard solution, prepared from 8 g. of bromobenzene and 2 g. of magnesium, is added an ether solution containing 7.00 g. of 2-phenylacetophenone-1-C^{14}. The reaction mixture is poured onto ice and dilute acid, and the product is extracted into ether solution, which is dried, concentrated and diluted with hexane; yield 6.40 g. (65%), m.p. 88–89° from hexane (Note 2).

(b) *1,1,2-Triphenylethylene-1-C^{14}* (Note 3). A mixture of 0.758 g. of 1,1,2-triphenylethanol-1-C^{14}, 8 ml. of water and 2 ml. of sulfuric acid is refluxed for 8 hours. The product is extracted into ether solution, which is dried and concentrated to yield 0.594 g. of crystalline material (84%). Purification is accomplished by recrystallization from alcohol; yield 0.504 g., m.p. 67–69°. The molar specific activity is 98.8% of that of the starting material, and degradation studies indicate that there is no isotopic rearrangement (Note 4).

(c) [*1,1,2-*]*Triphenylethylene-$C^{14}_{1/2}$*. A mixture of 0.500 g. of 1,1,2-triphenylethylene-1-C^{14} and 0.5 g. of phosphorus pentoxide (Note 5) is refluxed for 30 minutes with about 15 ml. of dry xylene. The cooled mixture is diluted with 100 ml. of water and extracted with 100 ml. of hexane. The extract is washed with water (until acid-free), dried and concentrated. Degradation studies indicate that the recovered olefin, m.p. 67–69°, has undergone 50:50 isomerization (Note 6).

B. Notes

1. The procedure is an adaptation of that of Klages;[1] also see Adkins and Zartman.[2]

2. Drying over phosphorus pentoxide for several days results in partial dehydration to the olefin.

3. The procedure is a modification of that of Adkins and Zartman.[2] By extending the reflux period 6 hours, the vacuum distillation (b.p. 215–225° at 15 mm.) is dispensed with, and a product of higher purity is obtained.

4. Permanganate oxidation of the olefin in the manner described below gives benzophenone-C^{14} 2,4-dinitrophenylhydrazone with the same molar specific activity.

5. When much less than an equal weight of phosphoric anhydride is used, only 27% of the isotope is found at C-2.

6. This permanganate oxidation procedure is typical of the degradations of various 1,1,2- and 1,2,2-triphenylethyl derivatives. There is evidence that about 1% phenyl migration occurs during the oxidation.

A mixture of 0.500 g. of triphenylethylene-$C^{14}_{1/2}$, 0.800 g. of potassium permanganate, 25 ml. of acetone, 10 ml. of water and 1 ml. of glacial acetic acid is stored at room temperature for nearly 1 hour. The excess permanganate is decomposed with bisulfite, and the filtered, concentrated solution is made alkaline and continuously extracted with ether. The extract is concentrated, diluted with 5 ml. of alcohol and treated with a solution of 0.400 g. of 2,4-dinitrophenylhydrazine in 2 ml. of sulfuric acid and 1 ml. of water. The mixture is warmed on a steam-bath, and the benzophenone-C^{14} 2,4-dinitrophenylhydrazone is crystallized; yield 0.217 g. (31%). The molar specific activity of a sample recrystallized from dioxane, m.p. 238–239°, is 52.2% of that of the unrearranged olefin.

The alkaline solution is acidified and extracted with ether, yielding 0.108 g. of benzoic-C^{14} acid (45%). The molar specific activity of a sample recrystallized from water, m.p. 121–122°, is 50.8% of that of the unrearranged olefin.

C. Other Preparations

In a similar manner from bromobenzene-$C^{14}_{1/4}$ are prepared[3] 1-phenyl-$C^{14}_{1/4}$-1,2-diphenylethanol and phenyl-$C^{14}_{1/4}$-diphenylethylene. The dehydration of 1-phenyl-$C^{14}_{1/4}$-2,2-diphenylethanol and 1,2,2-triphenylethanol-1-C^{14}, by boiling with xylene in the presence of anhydrous *p*-toluenesulfonic acid, gives 1,2-bis(phenyl-$C^{14}_{1/4}$)-2-phenylethylene, m.p. 68–70° (60.2% activity at 2-phenyl), and [1,2,2-]triphenylethylene-$C^{14}_{1/2}$ (47.9% activity at C-2). The same labeled olefins are obtained also by boiling the acetates with formic acid in the presence of *p*-toluenesulfonic

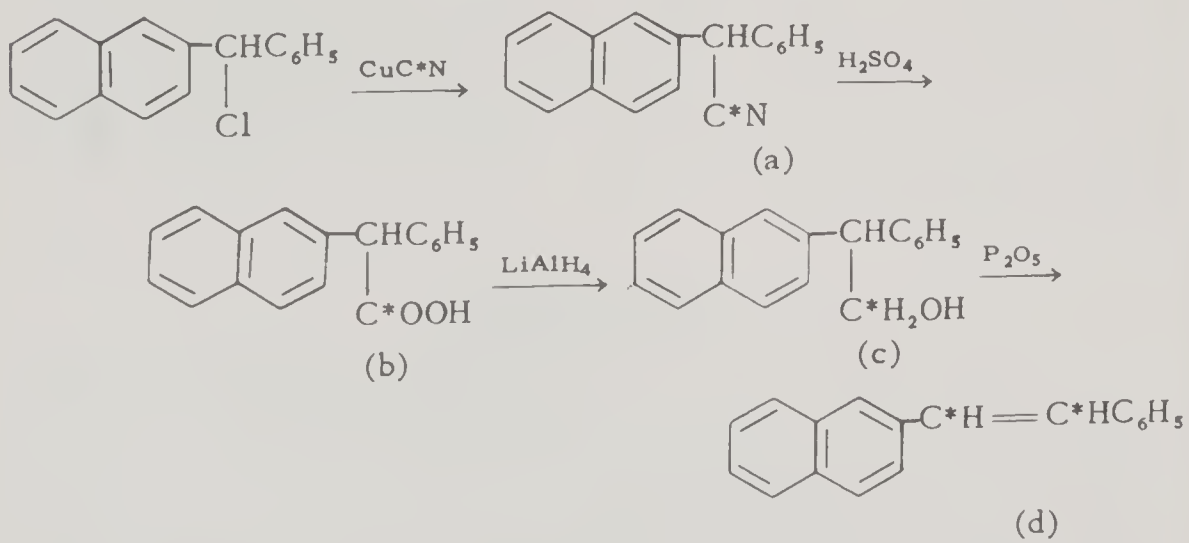
acid, giving the statistical isotopic redistribution: 66% activity at 2-phenyl and 50% activity at C-2, respectively. The same result is obtained when the ring-labeled carbinol or acetate is heated with formic acid. In the latter case there is also formed 1,2-bis(phenyl-$C^{14}_{1/4}$)-2-phenylethyl formate as by-product; yield 12%, m.p. 118–119° from alcohol-ligroin (66% activity at 2-phenyl). However, there is only 0.72% isomerization when a chain-labeled olefin[4] is heated with formic acid under the same conditions.

1,1,2-Triphenylethylene-1-C^{14}, m.p. 71–72°, has been prepared by dehydration of 1,1,2-triphenylethanol-1-C^{14} during simple recrystallization[5] from glacial acetic acid.

The preparation of [1,1,2-]triphenylethylene-$C^{14}_{1/2}$, by dehydration of 1,2,2-triphenylethanol-1-C^{14} with phosphoric anhydride in boiling xylene, has been described in detail by Bonner.[6] The product was isolated by chromatography on alumina and crystallized from acetic acid; yield 71%, m.p. 63–66°. Degradation of the recrystallized product indicates that one-half of the activity is located at C-2.

[1]A. Klages and S. Heilmann, Ber., 37, 1447 (1904).
[2]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 606.
[3]C. J. Collins and W. A. Bonner, J. Am. Chem. Soc., 77, 92 (1955).
[4]The preparation of 1,1,2-triphenylethylene-2-C^{14} will be published later by Collins and Bonner.
[5]J. F. Lane and D. R. Walters, J. Am. Chem. Soc., 73, 4234 (1951).
[6]W. A. Bonner and C. J. Collins, J. Am. Chem. Soc., 75, 5372 (1953).

2-STYRYL-α,β-$C^{14}_{1/2}$-NAPHTHALENE
[1-(2-Naphthyl)-2-phenylethylene-$C^{14}_{1/2}$]



C. J. Collins, L. S. Ciereszko and J. G. Burr, Jr., J. Am. Chem. Soc., 75, 405 (1953).

A. Procedure

(a) *α-Phenyl-2-naphthaleneacetonitrile-C^{14}.* The crude 2-(α-chlorobenzyl)naphthalene prepared from 12 g. of α-phenyl-2-naphthalenemethanol (Note 1) is mixed with 3.0 g. of cuprous cyanide-C^{14}, and the reaction flask is immersed in a preheated oil-bath at 200°. The mixture is heated for 2 hours at 200–210°; then the cooled melt is extracted with acetone, and the product is distilled under high vacuum. The thick yellow oil is dissolved in acetone, a white amorphous residue is filtered out, and from the filtrate is obtained 4.0 g. (32%) of an oil which crystallizes upon standing (2 days). Recrystallization from hexane yields colorless prisms, m.p. 85–86°.

(b) *α-Phenyl-2-naphthaleneacetic-C^{14} Acid.* α-Phenyl-2-naphthaleneacetonitrile-C^{14} (3.2 g.) is hydrolyzed by refluxing with 40 ml. of 50% sulfuric acid for 4 hours. The product is extracted with ether and then with 2 *N* sodium hydroxide. Acidification of the alkaline solution yields a tan-colored oil which subsequently crystallizes. Decolorization with Norit, crystallization from alcohol and recrystallization from methanol yield 1.65 g. of needles (50%), m.p. 128–130°.

(c) *β-Phenyl-2-naphthaleneëthanol-α-C^{14}.* α-Phenylnaphthaleneacetic-C^{14} acid (1.1 g.) is reduced to the carbinol by the action of 3 g. of lithium aluminum hydride in ether solution. The excess reagent is destroyed with ethyl acetate and sodium hydroxide. Ether extracts of this mixture yield 1.1 g. (94% yield) of an oil which, in time, crystallizes. Recrystallization from hexane yields needles, m.p. 63–64°.

(d) *2-Styryl-α,β-$C^{14}_{1/2}$-naphthalene.* β-Phenyl-2-naphthaleneëthanol 0.800 g.) is dehydrated and rearranged with 2 g. of phosphorus pentoxide and 2.5 ml. of xylene by the procedure described[1] for the preparation of benz[a]anthracene-5,6-$C^{14}_{1/2}$ by the Wagner rearrangement. The yield of product crystallized from methanol is 0.370 g. (50%), m.p. 146–147°. 2-Naphthyl migration occurs to the extent of 55.6%, as indicated by degradation studies (Note 2).

B. Notes

1. α-Phenyl-2-naphthalenemethanol, prepared[2] by reducing 2-naphthyl phenyl ketone, is treated with thionyl chloride; see 4-methoxystilbene-α, α′-$C^{14}_{1/2}$. The crude product has a purity of 80% based on chlorine analysis.

2. Directions are given by Collins for oxidizing the compound, with permanganate, to 2-naphthoic-C^{14} acid and benzoic-C^{14} acid. Determination of the migration ratio is not dependent upon separation of these two acids, but merely upon the ratio of the molar specific activities of the 2-naphthoic acid and the carbinol (c).

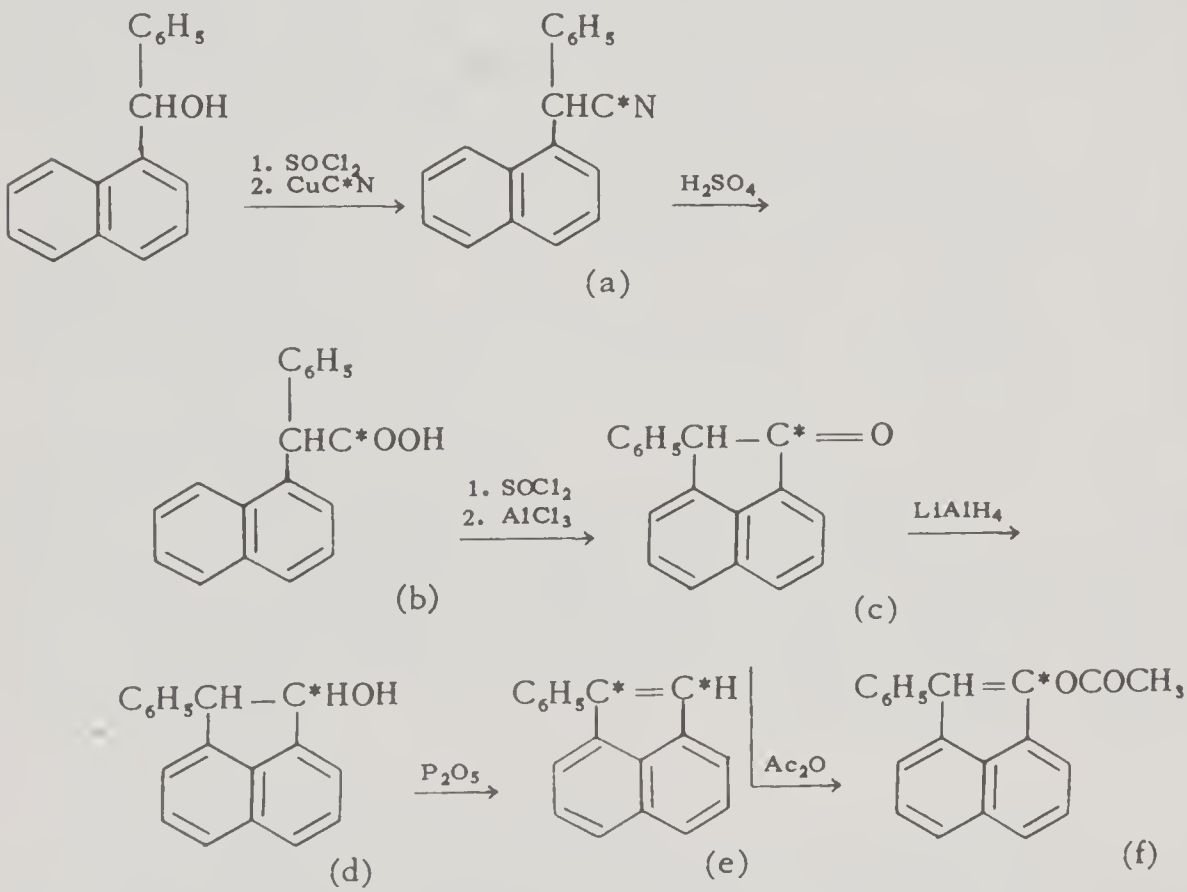
C. Other Preparations

By the same procedures are prepared: α-phenyl-1-naphthaleneacetonitrile-C^{14} (52%, m.p. 95–96°), α-phenyl-1-naphthaleneacetic-C^{14} acid (92%, m.p. 140–141°), β-phenyl-1-naphthaleneëthanol-α-C^{14} (42%, m.p. 91–92°), 1-styryl-α, β-$C^{14}_{1/2}$-naphthalene (100%, m.p. 69–71°). and 1-naphthoic-C^{14} acid (35%, m.p. 159–160°). The 1-naphthyl migration occurs to the extent of 52.3%.

[1]C. J. Collins, J. G. Burr, Jr. and D. N. Hess, J. Am. Chem. Soc., 73, 5176 (1951).

[2]J. F. Norris and J. T. Blake, J. Am. Chem. Soc., 50, 1808 (1928).

1-PHENYLACENAPHTHYLENE-1,2-$C^{14}_{1/2}$



W. A. Bonner and C. J. Collins, J. Am. Chem. Soc., 75, 2308, 3831 (1953).

A. Procedure

(a) α-*Phenyl-1-naphthaleneacetonitrile-*C^{14} (Note 1). α-Phenyl-1-naphthalenemethanol (125.5 g., Note 2) is refluxed for 1.5 hours with 200 ml. of thionyl chloride; then the excess of the latter is removed under vacuum at 100°. The dark, crude 1-(α-chlorobenzyl)naphthalene is

dissolved in 250 ml. of benzene, and the solution is distilled to dryness under vacuum. To the residue is added 48.4 g. (1 equiv.) of cuprous cyanide-C^{14}, and the mixture is heated at 100–210° (Note 3) for 2 hours with occasional swirling. An acetone solution of the melt is clarified by filtration with Celite and evaporated to dryness, giving 137 g. of crude product, 104%, (Note 4).

(b) *α-Phenyl-1-naphthaleneacetic-C^{14} Acid* (Note 1). The crude α-phenyl-1-naphthaleneacetonitrile-C^{14} is stirred vigorously for 4 hours with 500 ml. of 50% sulfuric acid under conditions of reflux. The mixture is extracted four times with a total of 600 ml. of hot benzene; cooling brings about crystallization of 58 g. of crude product, m.p. 108–112°. The mother liquor is extracted twice with hot 20% sodium hydroxide solution, and the alkaline extract is acidified and extracted with ether, the extract is washed with water, dried and decolorized with carbon. Solvent removal leaves 76 g. of amber-colored syrup, giving a combined crude yield of 99%. The combined acid is recrystallized from 240 ml. of a 1:1.4 methanol-benzene mixture, and successive crops are collected such that the yield of purified product totals 113.2 g., 81% (Note 5).

(c) *2-Phenyl-1-acenaphthenone-1-C^{14}* (Note 6). α-Phenyl-1-naphthaleneacetic-C^{14} acid (96.5 g.) is refluxed for 2 hours with 200 ml. of thionyl chloride; then the excess reagent is removed by distillation (finally at 2 mm.). The dark, residual α-phenyl-1-naphthaleneacetyl-C^{14} chloride (90.3 g.) is dissolved in 200 ml. of dry benzene, and the solution is added dropwise, with stirring over a 20-minute period, to a refluxing mixture of 300 ml. of benzene and 55 g. of anhydrous aluminum chloride. Stirring and heating are continued for 1 hour; then the mixture is cooled and treated with ice and dilute hydrochloric acid. The benzene extract is washed, dried and freed of solvent, and the 101.5 g. of dark oil is fractionally distilled at 2 mm. The fraction distilling at 150–190° (55 g.) is crystallized from a mixture of ether and ligroin, and the crude product (27.5 g., 31%) is purified by two recrystallizations from a 1:10 acetone-ligroin mixture; m.p. 115–116.5° (Note 7).

(d) *2-Phenyl-1-acenaphthenol-1-C^{14}*. A solution of 16.0 g. of 2-phenyl-1-acenaphthenone-1-C^{14} in 500 ml. of ether is added dropwise with stirring to a mixture of 1.0 g. of lithium aluminum hydride in 50 ml. of ether. After being stirred under reflux for 20 additional minutes, the excess hydride is destroyed by dropwise addition of excess ethyl acetate, followed by water and dilute sulfuric acid. The crude product (8.5 g.), m.p. 165.5–166.5°, is collected by filtration. The ether layer of the filtrate is separated, washed, dried and evaporated to give 8.0 g. of a semisolid, which, upon crystallization from a mixture of acetone and ligroin, gives 1.6 g. of product, m.p. 160–162° (Note 8). The combined product is purified by recrystallization from a mixture of 150 ml. of acetone and 250 ml. of ligroin; yield 7.8 g., m.p. 167.2–167.7°. Con-

centration of the mother liquor to 100 ml. yields 1.7 g. of additional product, m.p. 166.5–167.5°. Degradation studies indicate that there is no isotopic rearrangement (Note 9).

(e) *1-Phenylacenaphthylene-1,2-$C^{14}_{1/2}$* (Note 10). To a solution of 1.5 g. of 2-phenyl-1-acenaphthenol-1-C^{14} in 50 ml. of hot, dry benzene is added 0.75 g. of phosphorus pentoxide under the dehydrating conditions of the Wagner rearrangement. The mixture is boiled with swirling for 3 minutes, then cooled and washed twice with water, and the solvent is evaporated, leaving 1.47 g. of syrup. Extraction with 15 ml. of boiling alcohol leaves 0.60 g. of polymeric residue. The extract is treated with a solution of 0.9 g. of picric acid in 8 ml. of alcohol and cooled to 0°, yielding 0.37 g. of 1-phenylacenaphthylene-1,2-$C^{14}_{1/2}$ picrate, m.p. 121–123°. A benzene solution of the picrate is decomposed on a small column of alumina. Evaporation of the effluent and washings leaves 0.20 g. of oil which readily crystallizes upon seeding. The recrystallized product melts at 54–55°. Degradation studies (Note 11) indicate a phenyl migration to the extent of 7.5%.

(f) *2-Phenyl-1-acenaphthylenyl-1-C^{14} Acetate.* 2-Phenyl-1-acenaphthenone-1-C^{14} (0.50 g.), dissolved in 3 ml. of pyridine, is treated with 2 ml. of acetic anhydride and allowed to stand for 2 hours. The reaction mixture is diluted with excess water and allowed to stand 15 minutes, and the orange oil is extracted into ether. The extract is washed with water, dilute hydrochloric acid, water and sodium bicarbonate solution. Evaporation of solvent leaves a quantitative yield of an orange syrupy product (Note 12).

B. Notes

1. For a number of similar preparations by this method see 2-styryl-α, β-$C^{14}_{1/2}$-naphthalene and 4-methoxystilbene-α,α′-$C^{14}_{1/2}$. The small-scale isotopic synthesis had been described by Collins.[1]

2. Directions are given by Bonner and Collins for preparing the starting material by reduction of 1-naphthyl phenyl ketone with lithium aluminum hydride.

3. The oil-bath is preheated to 200°.

4. Collins[1] has reported a 52% yield of vacuum-distilled product, m.p. 95–96°, from hexane.

5. The maximum m.p. was 138.5–139.5°; no melting point was given for the last crop taken. Collins[1] has reported a yield of 92%, m.p. 140–141°, at the 10-mmole level.

6. The procedure is a modification of the method of Koelsch.[2]

7. The mother liquor, on prolonged standing, deposits a small amount of what appears to be the aldol of the product, m.p. 239–241°. The

preparation of this material, by the action of sodium ethoxide on the ketone, is described by Bonner and Collins.

8. Evaporation of the mother liquors produces 5.6 g. of syrup which, when dissolved in acetone-ligroin and allowed to stand, yields about 1 g. of the aldol, identical with the by-product isolated in the cyclization.

9. Directions are given by Bonner and Collins for oxidizing the carbinol or ketone to 8-benzoyl-1-naphthoic-C^{14} acid, m.p. 130.5°, with neutral permanganate in dilute acetone. Decarboxylation in quinoline over copper chromite forms nonisotopic 1-naphthyl phenyl ketone. There is a pronounced isotope effect in this oxidation of the carbinol, but with the intermediate ketone there is none. 2-Phenyl-1-acenaphthenyl-1-C^{14} acetate, m.p. 122.5–123° from alcohol, is formed with acetic anhydride; see (f).

10. The preparation results from an attempt to extend the study of the Wagner rearrangement mechanism to the *cis*-and *trans*-isomers of 2-phenyl-1-acenaphthenol-1-C^{14}. However, only one form was obtained by lithium aluminum hydride reduction of the ketone, and catalytic reduction of the ketone and the enol acetate.

11. Directions are given by Bonner and Collins for converting the olefin to 8-benzoyl-$C^{14}_{1/1}$-1-naphthoic-$C^{14}_{1/1}$ acid by ozonization[3] in acetone solution, followed by permanganate oxidation of the ozonide. Decarboxylation of the acid, over copper chromite in quinoline, gave barium carbonate-C^{14} and 1-naphthyl phenyl ketone-C^{14}, the latter assaying 7.5% of the molar specific activity of the starting material.

12. Directions are given by Bonner and Collins for characterization of the compound: a) the picrate melts at 145–146°; b) degradation by ozonization and alkaline hydrolysis of the ozonide gives a good yield of 8-benzoyl-1-naphthoic-C^{14} acid, m.p. 131–132°; c) hydrogenation over Raney nickel forms 1-phenylacenaphthene-2-C^{14}, m.p. 102–102.5°.

C. Other Preparations

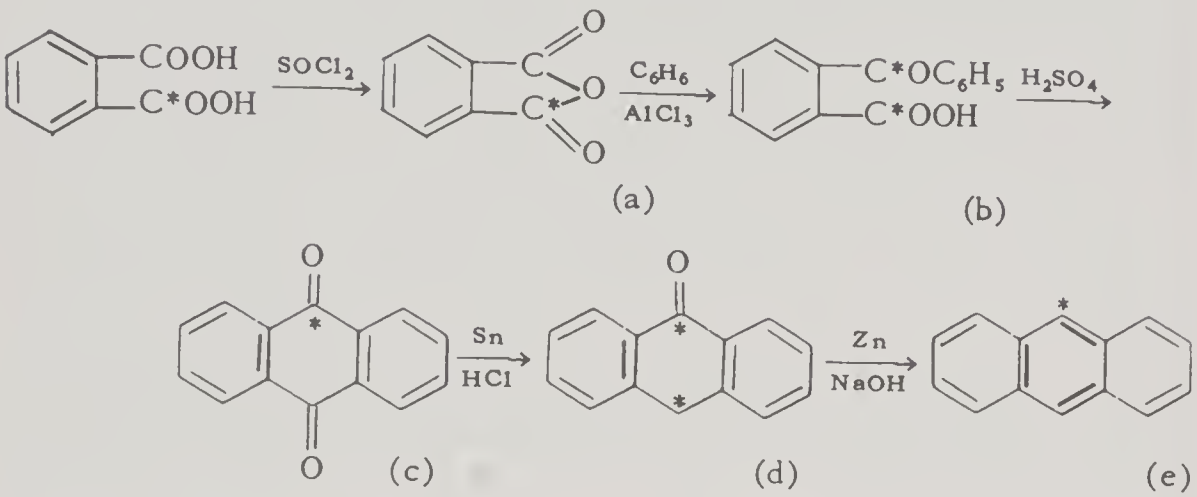
2-Phenyl-1-acenaphthenol-1-C^{14} has been prepared[4] by hydrogenation of 2-phenyl-1-acenaphthenone-1-C^{14} in alcohol over Adams catalyst. The yield was somewhat lower, and some 1-phenylacenaphthene-2-C^{14}, m.p. 103–104°, was formed when Raney nickel was used. The acetate of the same stereoisomer of the carbinol, along with some 1-phenylacenaphthene-2-C^{14}, was formed[4] by reduction of 2-phenyl-1-acenaphthenone-1-C^{14} enol acetate over Adams catalyst; only the second compound was obtained with Raney nickel.

[1]C. J. Collins, L. S. Ciereszko and J. G. Burr, Jr., J. Am. Chem. Soc., 75, 405 (1953).

[2]C. F. Koelsch and H. J. Richter, J. Am. Chem. Soc., 59, 2165 (1937).

[3]W. A. Bonner, J. Chem. Educ., *30*, 452 (1953).
[4]W. A. Bonner and C. J. Collins, J. Am. Chem. Soc., *75*, 3831 (1953).

ANTHRACENE-9-C^{14}



W. H. Stevens and D. A. Holland, Science, *112*, 718 (1950).

A. Procedure

(a) *Phthalic-C^{14}_1 Anhydride.* Phthalic-C^{14}_1 acid is refluxed for 1 hour with twice its weight of thionyl chloride. Three successive portions of benzene are distilled from the phthalic anhydride to free it from excess dehydrating agent.

(b) *o-Benzoyl-$C^{14}_{1/1}$-benzoic-$C^{14}_{1/1}$ Acid.* The keto acid is prepared from the phthalic-C^{14}_1 anhydride by a Friedel-Crafts reaction using 10 ml. of benzene and 3.8 g. of aluminum chloride. The crude acid is dissolved in dilute ammonium hydroxide and clarified by filtration with diatomaceous earth. Acidification with hydrochloric acid precipitates the product as an oil, which crystallizes upon standing.

(c) *Anthraquinone-9-C^{14}.* The ring closure is effected by dissolving the o-benzoyl-$C^{14}_{1/1}$-benzoic-$C^{14}_{1/1}$ acid in 30 ml. of 96% sulfuric acid and heating at 120° for 1 hour (Note 1). The reaction mixture is poured into an excess of cold water, and the slurry is digested on a hot plate. The product is collected, washed with warm, dilute ammonium hydroxide and dried at 120°; yield 2.1 g.

(d) *Anthrone-9,10-$C^{14}_{1/2}$.* A mixture of the anthraquinone-9-C^{14}, 5 g. of mossy tin and 50 ml. of glacial acetic acid is refluxed while, in the course of 1.5 hours, concentrated hydrochloric acid is added in small portions until the diketone dissolves. The solution is diluted with 25 ml. of water, filtered hot, and stored overnight in a refrigerator. The crystallized product is collected and washed with water (Note 2).

(e) *Anthracene-9-C*^{14}. A mixture of the anthrone-9,10-$C^{14}_{1/2}$, 80 ml. of 2 *N* sodium hydroxide, 20 ml. of toluene and 10 g. of activated zinc dust (Note 3) is refluxed for 24 hours in a 200-ml. round-bottomed flask. Hot benzene (20 ml.) is added, and the liquid is decanted into a separatory funnel, the zinc being washed with another 20 ml. of hot benzene. The benzene-toluene layer is separated and decolorized with 1 g. of carbon, and the solvent is evaporated in a jet of nitrogen. The residue is crystallized from 15 ml. of toluene as colorless, fluorescent flakes, m.p. 215°; yield 1.3 g. (Note 4).

B. Notes

1. The extent of reaction is about 10% when a solution of 1.4 g. of *o*-benzoylbenzoic-C^{14} acid in 10 ml. of sulfuric acid is heated at 80 ± 0.5° for 15 minutes,[1] but is nearly quantitative when the mixture is heated at 100° for 3 hours. A reverse isotope effect in the condensation of this acid to anthraquinone-9-C^{14} has been reported by Stevens,[1] but could not be confirmed by Ropp[2] who has discussed possible sources of error.

2. Meyer[3] found the yield to be 82.5% on a large scale, m.p. 153°.

3. To 10 g. of zinc dust is added 10 ml. of aqueous solution containing 0.04 g. of $CuSO_4 \cdot 5H_2O$. After a few minutes the activated zinc is washed once with water by decantation.

4. Martin[4] has reported a 93% yield of anthracene by this reaction. The reduction of anthraquinone directly by this reaction gives only 80% yields of anthracene of "inferior fluorescence."

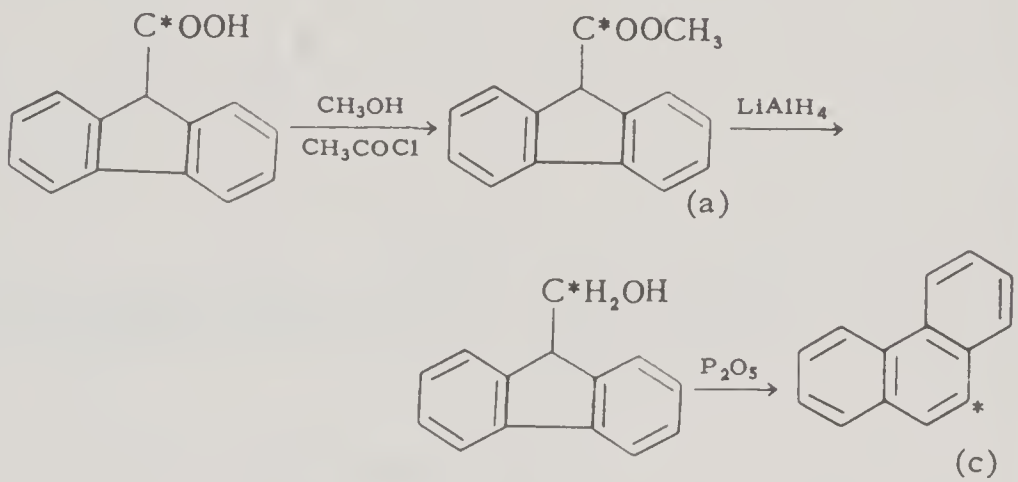
C. Other Preparations

The preparation of benz[*a*]anthracene-7,12-dione-7,12-$C^{14}_{1/2}$ by a similar procedure, at the 3-mmole level, has been reported by Hadler.[5] Phthalic-C^{14}_1 acid was converted to phthalic-C^{14}_1 anhydride by sublimation;[6] m.p. 126–130°, yield 89% based on o-toluic acid. The anhydride was condensed with naphthalene by the Friedel-Crafts procedure in ethylene chloride (recommended for α-acylations[7]), the complex was decomposed with hydrochloric acid, and the solvent was removed by steam distillation, giving a quantitative yield of crude o-1-naphthoyl-$C^{14}_{1/1}$-benzoic-$C^{14}_{1/1}$ acid, m.p. 110–130°. The ring closure was accomplished[8] by heating at 130° with concentrated sulfuric acid, and the benz[*a*]anthracene-7,12-dione-7,12-$C^{14}_{1/2}$ was purified by chromatography on Florisil (60/100), with benzene as eluant; yield 60%, m.p. 167.5–168°. The quinone was converted to 7,12-dimethylbenz[*a*]-anthracene-7,12-$C^{14}_{1/2}$ (yield 46%, m.p. 120–121°) by the method of Sandin,[9] through condensation with methylmagnesium iodide, decomposition of the complex with hydriodic acid (sp. gr. 1.7) in methanol, and reduction of the resulting 7-iodomethyl-12-meth-

ylbenz[*a*]anthracene-7,12-$C^{14}_{1/2}$ with stannous chloride in dioxane and concentrated hydrochloric acid. Purification was accomplished by chromatography on Florisil (semi-darkness), with cyclohexane as eluant; overall yield 18% based on barium carbonate.

[1]W. H. Stevens and D. A. Crowder, Can. J. Chem., *32*, 792 (1954).
[2]G. A. Ropp, J. Chem. Phys., *23*, 2196 (1955).
[3]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 60.
[4]E. L. Martin, J. Am. Chem. Soc., *58*, 1438 (1936).
[5]H. I. Hadler, J. Am. Chem. Soc., *77*, 1052 (1955).
[6]W. Werth, Ber., *7*, 1057 (1874).
[7]G. Baddiley, J. Chem. Soc., *1949*, S59.
[8]G. M. Badger and J. W. Cook, J. Chem. Soc., *1939*, 802.
[9]R. B. Sandin and L. F. Fieser, J. Am. Chem. Soc., *62*, 3098 (1940).

PHENANTHRENE-9-C^{14}



C. J. Collins, J. Am. Chem. Soc., *70*, 2418 (1948).

A. Procedure

(a) *Methyl 9-Fluorenecarboxylate-*C^{14}. 9-Fluorenecarboxylic-C^{14} acid (0.1220 g.) is allowed to stand for 1 hour at room temperature in a solution of 5 ml. of methanol and 0.15 ml. of acetyl chloride. The solution is then evaporated in an air stream and dried to constant weight over phosphorus pentoxide; yield 0.1224 g. (Note 1).

(b) *9-Fluorenemethanol-*α-C^{14}. To a solution of the methyl 9-fluorenecarboxylate-C^{14} in 7 ml. of ether is added 10 ml. of 0.16 *M* lithium aluminum hydride solution (ether). After 15 minutes of stirring 5 ml. of moist ether is added, followed by 5 ml. of 6 *N* hydrochloric acid. The ether layer is separated, washed with 5 ml. of water and evaporated to dryness; yield 0.1148 g. of oil (Note 2).

(c) *Phenanthrene-9-*C^{14}. The 9-fluorenemethanol-α-C^{14} is dehydrated under the conditions of the Wagner rearrangement, by refluxing for 30

minutes with 10 ml. of dry xylene and 0.20–0.40 g. of phosphorus pentoxide. After cooling, 5 ml. of water is added, and the xylene layer is separated, washed with two 5-ml. portions of water, evaporated and dried, yielding 0.170 g. of oil. To this residue in a centrifuge cone is added 0.060 g. of picric acid and 3.0 ml. of saturated alcoholic picric acid solution. The mixture is heated to a clear solution and slowly cooled, then the picrate is separated by centrifuging and drawing off the supernatant liquor. The picrate is decomposed with dilute aqueous sodium hydroxide solution, and the mixture is extracted with ether. The extract is washed with water and evaporated to dryness. The crude product is purified by a repetition of this process; yield 0.0725 g., m.p. 94–95° (Note 3).

B. Notes

1. The yield in trial runs was 98–100%, m.p. 63.0–63.5°.

2. The crude product from a trial run melted at 80–85° (87% yield); after one recrystallization, m.p. 94–96°. Reduction of the free acid gives lower yields than does the ester.

3. Recrystallization from alcohol yields colorless crystals, m.p. 96–97°. The picrate melts at 141°, and there is no lowering upon mixed melting with an authentic sample. Trial runs have given yields of 90–94%.

Purification by chromatography on alumina (1 × 18 in.) has been described.[1] Development and elution with light petroleum (b.p. 60–80°) give the labeled product; yield 88% based on the carbinol, m.p. 93–95°.

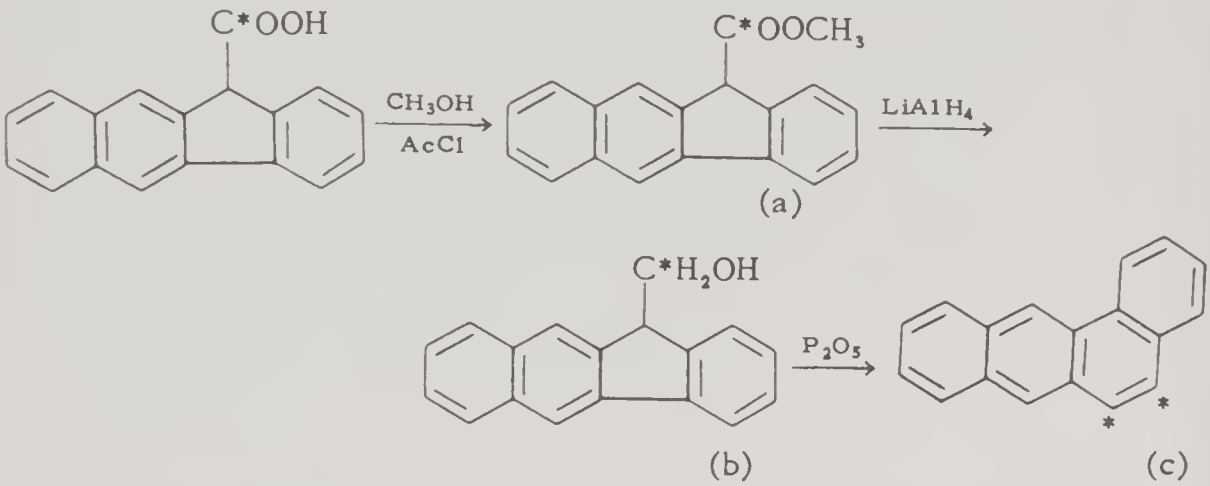
C. Other Preparations

In a similar manner from 3-methoxy-9-fluorenecarboxylic-C^{14} acid is prepared[2] the series: methyl 3-methoxy-9-fluorenecarboxylate-C^{14} (*via* diazomethane, yield 100%, m.p. 108–109° from ether), 3-methoxy-9-fluorenemethanol-α-C^{14}, 3-methoxyphenanthrene-9,10-$C^{14}_{1/2}$ (yield 93%, the oil does not readily crystallize; degradation studies indicate that 97.6% of the activity is at C-10). The oily ether is refluxed for 2 hours with 20 ml. of 55% hydriodic acid. The mixture is extracted with ether, and the ether solution is extracted with two 20-ml. portions of 4% sodium hydroxide containing a trace of sodium thiosulfate. Acidification, ether extraction and recrystallization from petroleum ether yield 3-phenanthrol-9,10-$C^{14}_{1/2}$ (yield 44.2%, m.p. 120–121°). Directions are given by Collins[2] for the permanganate oxidation of this compound to phthalic-C^{14}_{1} acid, which is converted to the dianilide for purification and assay. The molar specific activity is 2.4% of that of the starting material, indicating that this amount of activity is contributed by C-9.

[1] A. S. Harris, E. N. White and D. McNeil, J. Chem. Soc., *1955*, 4216.

[2] C. J. Collins and B. M. Benjamin, J. Am. Chem. Soc., 75, 1644 (1953).

BENZ[*a*]ANTHRACENE-5,6-$C^{14}_{1/2}$



C. J. Collins, J. G. Burr, Jr. and D. N. Hess, J. Am. Chem. Soc., 73, 5176 (1951).

Procedure

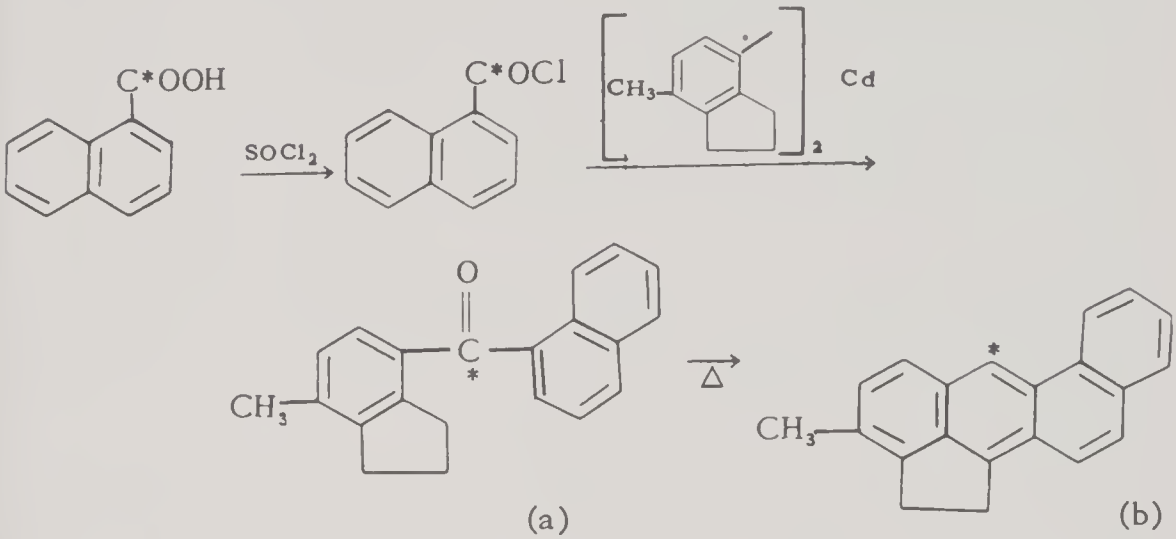
(a) *Methyl 11*H-*Benzo*[b]*fluorene-11-carboxylate-*C^{14}. To a solution of 0.0868 g. of 11*H*-benzo[*b*]fluorene-11-carboxylic-C^{14} acid in 10 ml. of methanol is added 5 drops of acetyl chloride. After 1 hour the mixture is evaporated to dryness; yield 0.0908 g. (100%), m.p. 117–118°.

(b) *11*H-*Benzo*[b]*fluorene-11-methanol-*α-C^{14}. To a solution of 0.078 g. of methyl 11*H*-benzo[*b*]fluorene-11-carboxylate-C^{14} in 15 ml. of ether is added a solution of 0.4 g. of lithium aluminum hydride in 20 ml. of ether, and stirring is continued for 20 minutes. The mixture is hydrolyzed with wet ether, followed by 6 *N* hydrochloric acid. The ether layer is washed with sodium carbonate solution and water, then dried and evaporated. The residue is dissolved in benzene, and the solution is passed through a small column of alumina. Repeated elution with benzene removes 0.0237 g. of yellow resin. Elution with absolute alcohol yields 0.0560 g. of product (80% yield), which solidifies to white crystals (m.p. 166–168°) after trituration with benzene.

(c) *Benz*[a]*anthracene-5,6-*$C^{14}_{1/2}$. A mixture of 0.030 g. of 11*H*-benzo[*b*]-fluorene-11-methanol-α-C^{14}, 10 ml. of dried xylene and 0.5 g. of phosphorus pentoxide is refluxed for 35 minutes, then hydrolyzed with 25 ml. of water. The product is isolated by continuous ether extraction (15 hours) and evaporation of the solvent. The solid residue (0.0281 g.) is dissolved in benzene and passed through a small alumina column; yield 0.0265 g. (93%) of an orange solid, m.p. 138–144°. One recrystallization from ethanol gives a colorless product melting at 156–157°; picrate, m.p. 137–138°. The molar specific activity is unchanged from that of the starting material, and degradation studies indicate that the activities of C-5 and C-6 are in the ratio of 48:52 (see 11-chrysofluorenone-11-C^{14}).

3-METHYLCHOLANTHRENE-6-C^{14}

METHOD I



W. G. Dauben, J. Org. Chem., *13*, 313 (1948).

A. Procedure

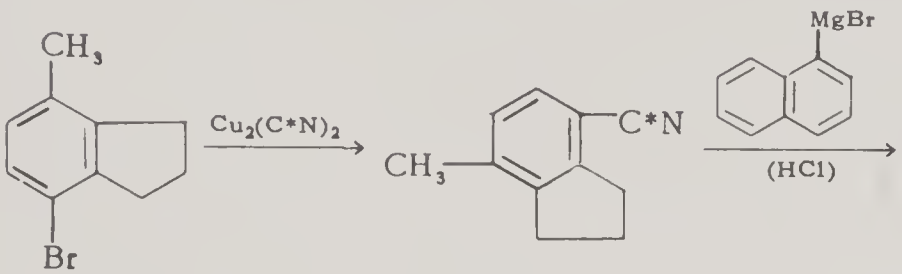
(a) *7-Methyl-4-indanyl 1-Naphthyl Ketone-C^{14}.* 1-Naphthoic-C^{14} acid (2.480 g., 14 mmoles) is refluxed with 10 ml. of purified thionyl chloride for 2 hours. The excess reagent is removed under reduced pressure, the 1-naphthoyl-C^{14} chloride is dissolved in dry benzene, and the solvent is distilled; after repetition of this process, the residue is dissolved in 10 ml. of benzene.

A mixture of 12.3 g. (58 mmoles) of 4-bromo-7-methylindan, 1.46 g. (60 mmoles) of magnesium, 50 ml. of absolute ether, 20 ml. of benzene and a few drops of ethyl iodide is refluxed for 24 hours in a nitrogen atmosphere. The Grignard reagent is converted to bis(7-methyl-4-indanyl)-cadmium by adding 6.4 g. (35 mmoles) of powdered anhydrous cadmium chloride (Note 1) and refluxing for 1 hour. The solvents are distilled and 25 ml. of benzene is added. To the resulting suspension, heated under reflux, is added the benzene solution of 1-naphthoyl-C^{14} chloride, and heating is continued for 1 hour. The mixture is decomposed with dilute hydrochloric acid and steam-distilled to remove solvent and volatile by-products. The residue is extracted into ether, and the extract is washed with water, *N* sodium hydroxide and water, then dried. The yield of distilled product is 4.063 g. (98.7%), b.p. 223–225° (2mm.)(Note 2).

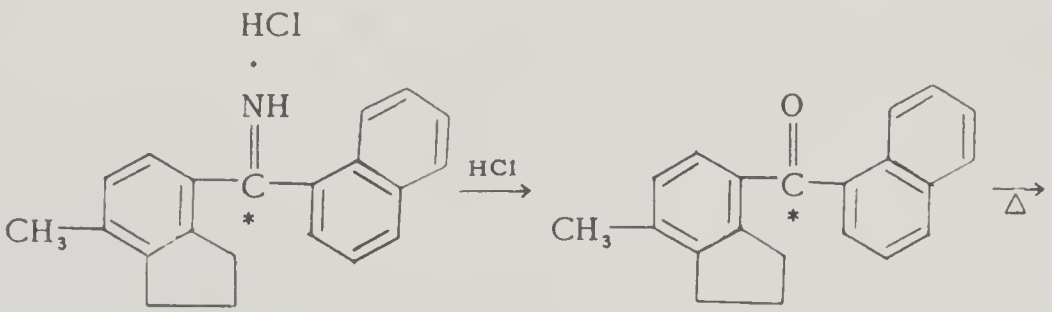
(b) *3-Methylcholanthrene-6-C^{14}.* The ketone (4.06 g., 14.5 mmoles) is pyrolyzed by heating in a slow stream of nitrogen for 40 minutes at 405–410° in a small distilling flask with a sealed-on receiver (Note 3). Glass wool is added, the top of the flask is sealed off, and the product

is collected by distillation at 1 mm. with a bath temperature of 350–400°. The yellow distillate is crystallized from 45 ml. of 1-propanol. The yield of product, recrystallized from 50 ml. of 1-propanol, is 1.436 g. (37.8%), m.p. 176.5–177.5°.

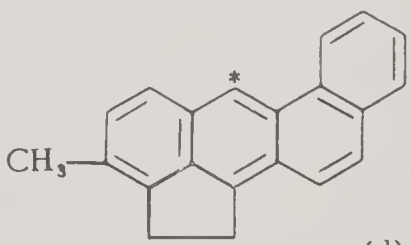
METHOD II



(a)



(b) (c)



(d)

L. Martin and S. B. Baker, Atomic Energy Commission Report, NP-3177; Nuc. Sci. Abstracts, 5, 5157 (1951).

A. Procedure (Note 4)

(a) *7-Methyl-4-indancarbonitrile-C*^{14}. To 0.065 g. of dry cuprous cyanide-C^{14} in a large Pyrex test tube is added a stoichiometric amount of 4-bromo-7-methylindan (Note 5) in 2 ml. of dry pyridine (Note 6). The tube is placed in an electric furnace so that at least 4 cm. protrudes above the furnace. The temperature is raised gradually to 210° over a period of 2 hours, then maintained at that level for 5 hours. The mixture is cooled and extracted with ether until no further color is extracted. The combined extract is concentrated, and the residue is distilled at 8 mm.

with a bath temperature of 180° (Note 7). The yield of crystallized product is 46% (Note 8).

(b) *α-(7-Methyl-4-indanyl)-1-naphthalenemethylenimine-α-C*^{14} *Hydrochloride.* A Grignard solution, prepared from 1 g. of 1-bromonaphthalene, in 5 ml. of ether is diluted to 10 ml. with anhydrous toluene. To a tube containing the carbonitrile is added an aliquot of the Grignard reagent containing 5 times the stoichiometric amount. The mixture is heated at 70°, with the exclusion of air, then cooled and decomposed with 6 ml. of 18% hydrochloric acid. After standing for 2 hours, the ketimine salt is collected and washed with water and ether.

(c) *7-Methyl-4-indanyl 1-Naphthyl Ketone-C*^{14}. The ketimine hydrochloride is hydrolyzed by refluxing for 3 hours with 1 ml. of hydrochloric acid, 1 ml. of acetic acid and 1 ml. of toluene (Note 9). The cooled mixture is extracted with 15 ml. of ether, and the extract is dried over sodium sulfate and concentrated. The residual oil is distilled (Note 7) at 8 mm. with a bath temperature of 300° (Note 10).

(d) *3-Methylcholanthrene-6-C*^{14}, (*20-Methylcholanthrene-11-C*^{14}) (Note 11). The ketone is pyrolyzed by the Elbs method,[1] by heating at 385° for 15 minutes in a nitrogen atmosphere (Note 7). The product is twice distilled at 8 mm. (Note 7) with a bath temperature of 270–300°, then crystallized from benzene-ether, 1:5 (Note 12); yield 0.02 g. (9.4%).

B. Notes

1. The salt is dried to constant weight at 110°. See Cason[2] for a discussion of this method for preparing ketones.

2. The product is prepared in 88.7% yield at the 3-mmole level by the alternate procedure of reacting di(1-naphthyl)cadmium with 7-methyl-4-indancarbonyl chloride. The latter acid is prepared in only 64% yield, whereas 1-naphthoic acid is prepared in 82% yield, by the Grignard reaction.

3. The evolved gases from the Elbs reaction contain considerable radioactivity (0.265 g. of barium carbonate-C^{14}).

4. The procedure is a modification of the method reported by Fieser and Seligman,[3] and modified by Cagniant.[4]

5. Directions are given by Fieser[5] and Cagniant[4] for preparing the starting material. *p*-Bromotoluene is converted by the Blanc reaction, with formaldehyde and hydrogen chloride, into a mixture of the two chloromethyl derivatives (substitution ortho to the bromine predominates in the ratio of 1.8 to 1). The mixture is treated with sodiummalonic ester, and the liquid product is hydrolyzed and decarboxylated. Ring closure through the acid chloride mixture ($SOCl_2$, $AlCl_3$) gives a mixture of two bromomethylhydrindones which, on reduction by the Clemmensen method, yield the same hydrindene, b.p. 265–267° (150° at 30 mm.).

6. A cuprous cyanide complex forms in pyridine with evolution of heat, and the subsequent reaction takes place at a lower temperature. Fieser and Seligman conducted the reaction in a sealed tube.

7. A Späth bulb is used in the distillation.

8. The pure compound melts at 72.9–73.2°, b.p. 162–164°. The amide, m.p. 176–177.4°, is formed by refluxing the nitrile with concentrated hydrochloric acid. Alkaline hydrolysis of the amide forms the acid, m.p. 227–229°.

9. The acetic acid facilitates the hydrolysis, while the toluene dissolves the ketone as it forms, and also floats the solid material, preventing bumping.

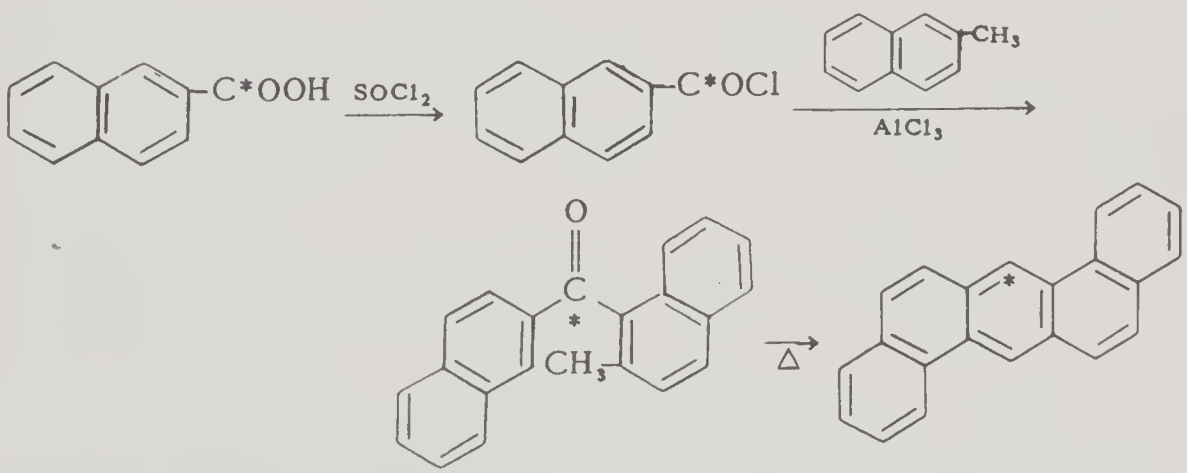
10. The pure compound distills at 211–214° (2 mm.).

11. The latter nomenclature is that of Fieser and of Cagniant.

12. Pure 3-methylcholanthrene crystallizes as yellow needles, m.p. 178.5–179.0° (cor.). The picrate occurs as purplish black needles, m.p. 182.0–182.5° (cor.).

[1]*Organic Reactions*, Vol. I, Wiley, New York, 1942, p. 129.
[2]J. Cason, J. Am. Chem. Soc., *68*, 2078 (1946).
[3]L. Fieser and A. M. Seligman, J. Am. Chem. Soc., *58*, 2482 (1936).
[4]P. Cagniant and D. Cagniant, Bull. soc. chim., France, (5) *15*, 1012 (1948).
[5]L. F. Fieser and A. M. Seligman, J. Am. Chem. Soc., *57*, 942 (1935).

DIBENZ[*a*,*b*]ANTHRACENE-7-C^{14}
(1,2,5,6-Dibenzanthracene-9-C^{14})



(a) (b)

C. Heidelberger, P. Brewer and W. G. Dauben, J. Am. Chem. Soc., *69*, 1389 (1947).

Procedure

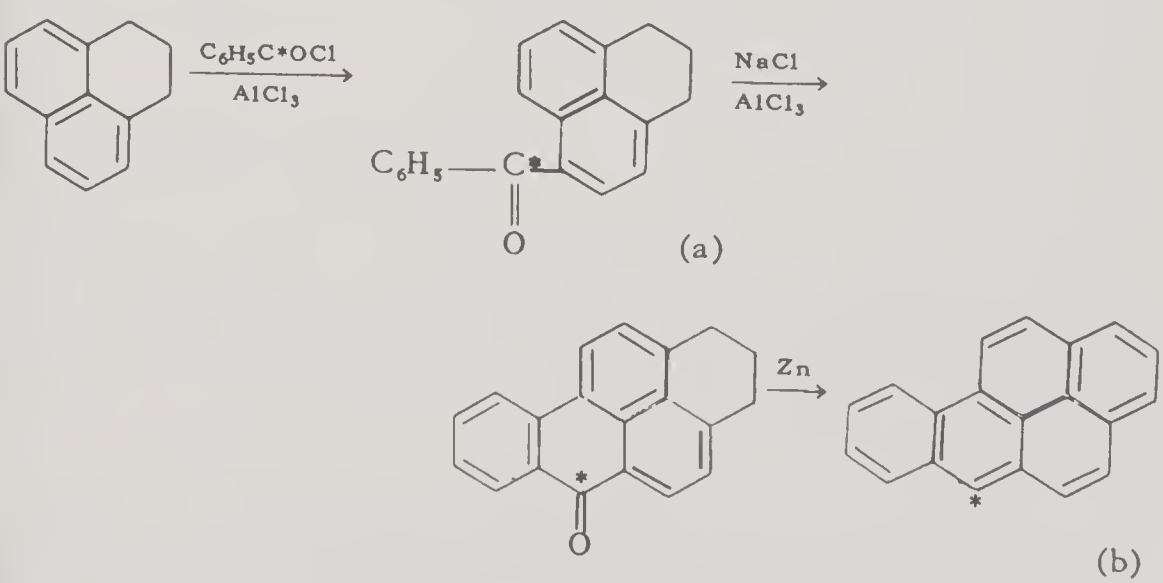
(a) *1-(2-Methylnaphthyl) 2-Naphthyl Ketone-C*^{14}. To a suspension of 0.95 g. of 2-naphthoic-C^{14} acid in 8 ml. of dry benzene is added 2 ml. of

purified thionyl chloride, and the mixture is refluxed for 1 hour. The excess reagent and solvent are removed by vacuum distillation. A mechanical stirrer is attached to the reaction flask, and a solution of redistilled 2-methylnaphthalene in 4 ml. of carbon disulfide is added, followed by 0.95 g. of anhydrous aluminum chloride over a period of 15 minutes. The mixture is refluxed for 1 hour, then hydrolyzed with dilute hydrochloric acid, and the solvent is removed by steam distillation. The product is extracted into benzene, washed with water and distilled. The yield of yellowish, viscous oil is 1.30 g. (80%), b.p. 212–215° (0.5 mm.).

(b) *Dibenz*[a,h]*anthracene-7-C*^{14}. The ketone (1.25 g.) is cyclodehydrated under the conditions of the Elbs reaction,[1] by heating at 435° for 3.5 hours in a small distilling flask with a sealed-on receiver. Glass wool is added, the top of the flask is sealed, and the product is distilled at 1 mm. (bath temperature 350–400°). The distillate is crystallized from hot benzene to give 0.330 g. of colored product, m.p. 231–243°. Purification is effected by boiling the product for 1 hour with a mixture of 0.07 g. of lead tetraacetate, 16 ml. of acetic acid and 16 ml. of benzene. The reaction mixture is concentrated (12 ml.), and the colorless product is filtered off and washed with cold acetic acid; yield 0.230 g., m.p. 249–253°. Recrystallization from benzene gives 0.207 g. of colorless plates, m.p. 259–262°, 18% yield. The preparation of radioautographs of the product is described by Grosse and Snyder.[2]

[1]*Organic Reactions*, Vol. I, Wiley, New York, 1942, p. 151.
[2]A. V. Grosse and J. C. Snyder, Science, *105*, 240 (1947).

BENZO[*a*]PYRENE-6-C^{14}



C. Heidelberger and H. S. Rieke, Cancer Research, *11*, 640, 251 (1951).

A. Procedure (Note 1)

(a) *2,3-Dihydro-1*(H)*-benzonaphthen-6-yl Phenyl Ketone-C*^{14}. To 1.126 g. of benzoyl-C^{14} chloride is added slowly 1.078 g. of fresh anhydrous aluminum chloride with warming; then the mixture is heated over a free flame until completely melted. The complex is dissolved in 15 ml. of carbon disulfide and added slowly, from a warmed dropping funnel, to a warm solution of 1.295 g. of perinaphthane (Note 2) in 5 ml. of carbon disulfide. The mixture is refluxed for 30 minutes, cooled and decomposed with ice and dilute hydrochloric acid. The solvent is evaporated, and the product is extracted into ether, washed with water and dried. The ether is distilled on a steam-bath, the residue is vacuum-distilled at 160–180° (10 μ) (Note 3), and the distillate is redistilled; yield 1.92 g. (89%) of light-yellow viscous oil (Note 4).

(b) *Benzo*[a]*pyrene-6-C*^{14}, (*1,2-Benzpyrene-3-C*^{14}), (3,4-Benzpyrene-5-C^{14}). A melt of 10.15 g. of freshly sublimed aluminum chloride and 2.92 g. of dry sodium chloride is poured onto 1.92 g. of the ketone in a 25-ml. Erlenmeyer flask. The flask is loosely stoppered and heated in an oil-bath for 3 hours at 130–140°, then for 2 hours at 145–150°. The mixture is decomposed with ice and dilute hydrochloric acid (Note 5), and the brown, gummy precipitate is collected and washed with water (Note 6). The crude solid and the filter paper are macerated and thoroughly mixed with 31.8 g. of zinc dust in a distillation flask (Note 7). The mixture is distilled in about 20 minutes by heating at over 600° with a sodium-potassium nitrate bath (Note 8). The orange-brown distillate is dissolved in a small amount of benzene and chromatographed in darkness, without delay, on a 2.5 × 40-cm. column of activated alumina. With occasional brief inspections under ultraviolet light (Note 9), the column is eluted with 1:1 purified benzene-ligroin. A band of yellow-fluorescent impurity is eluted, followed by a blue-fluorescent band of product which is collected (Note 10). The solvent is removed in darkness by evaporation under vacuum at 35°, leaving 0.745 g. (42%) of crude product, m.p. 168–171°. The final purification is carried out on 30-mg. batches in 10% benzene in ligroin (as needed) by chromatography in darkness on a 1 × 20-cm. column of activated alumina. The product fraction is cut sharply, and the solvent is evaporated under vacuum. The residue is dissolved in ethanol, and the product is precipitated by treatment with water, collected and dried. The faintly yellow product, m.p. 177–178°, is obtained in 70% recovery.

B. Notes

1. The procedure is a modification of the method of Fieser and Hershberg.[1]

2. Directions are given by Heidelberger for preparing the starting material by the catalytic hydrogenation of 7-perinaphthanone[2] over palladium-charcoal.

3. The distillation is by short-path.

4. The product melts at 62–63° after crystallization from ether-petroleum ether.

5. Considerable effort is required to effect complete decomposition of gummy solid resulting from the Scholl cyclization.

6. Experiment has shown that the small amount of dark brown oil in the filtrate is not converted into benzpyrene.

7. The distillation flask is fitted with a wide-path, sealed-on receiver cooled with wet cotton.

8. The yield is decreased by distilling too slowly, or at too low a temperature. Neither further heating, nor vacuum distillation increase the yield.

9. The product is sensitive to photo-oxidation. The solvents are washed with concentrated sulfuric acid and water, then dried over sodium sulfate and distilled from sodium.

10. A large residual band of yellow-fluorescent impurities is eluted with alcohol. The two yellow impurities may be recovered from the solvents and redistilled from zinc dust to yield an additional small amount of product.

[1]L. F. Fieser and E. B. Hershberg, J. Am. Chem. Soc., *60*, 1658 (1938).
[2]L. F. Fieser and M. D. Gates, Jr., J. Am. Chem. Soc., *62*, 2335 (1940).

BROMOMETHANE-C^{14}
(Methyl-C^{14} Bromide)

METHOD I

$$C^*H_3OH \xrightarrow{PBr_3} C^*H_3Br$$

B. Riegel and F. S. Prout, J. Org. Chem., *13*, 933 (1948).

A. Procedure (Note 1)

To 0.23 ml. of phosphorus tribromide, frozen with liquid nitrogen in a 45-ml. evacuated flask (3 μ), is added 2.13 mmoles of methanol-C^{14} by distillation. The flask is closed by stopcock, and the mixture is stored at room temperature (30°) for 4 hours. The product is vacuum-distilled slowly at 0° through a tube packed with porcelain chips (soaked in concentrated sulfuric acid) and sodium hydroxide pellets, and is condensed in a storage bulb with liquid nitrogen; yield approximately quantitative (Note 2).

METHOD II

$$C^*H_3OH \xrightarrow[H_2SO_4]{HBr} C^*H_3Br$$

W. W. Foreman, A. Murray III, and A. R. Ronzio, J. Org. Chem., *15*, 119 (1950).

A. Procedure (Note 3)

The apparatus consists of a heavy-walled flask fitted with a sealed-on reflux condenser, a pressure stopcock and a magnetic stirring bar (Note 4). The flask containing 5 ml. of an acid mixture, prepared by the dropwise addition of 3.6 ml. of concentrated sulfuric acid to 23.0 g. of chilled 48% hydrobromic acid, is evacuated briefly to remove dissolved gases, then is cooled in liquid nitrogen and evacuated to 0.1 μ (Note 5). Methanol-C^{14} (0.8–10 mmoles) is vacuum-distilled into the reaction flask, which then is closed off from the manifold. The stirred mixture is warmed to room temperature, and gradually heated to 100° over a period of 75 minutes, then cooled to room temperature. The product is distilled slowly through a purifying train, containing soda lime and phosphorus pentoxide-on-glass wool (see methyl-C^{14} iodide, Method I), into an evacuated storage flask, fitted with a pressure microstopcock. Quantitative transfer is achieved by cooling with liquid nitrogen, and any noncondensable gas is pumped off; yield 94–96% (Note 6).

B. Notes

1. The procedure is an adaptation of the method of Tolbert; see methyl-C^{14} iodide, Method II.

2. The amounts of methanol and product are determined manometrically by expanding the material into a vacuum of known volume (505 ml.) at 30°.

3. The procedure is a modification of the method of Kamm and Marvel,[1] which is general for the preparation of alkyl halides from primary alcohols.

4. The original paper contains a diagram of the system. A convenient reaction flask is that described for the preparation of iodomethane-C^{14}, Method I.

5. An HBr-H_2SO_4 reagent having a mole ratio of 1:1.55 gave slightly lower yields than did the one used (1:2.06).

6. The purity was checked by gravimetric bromine analysis of a trial preparation, by digestion for 48 hours with alcoholic silver nitrate solution at 45° in a sealed flask.

[1] *Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 25.

IODOMETHANE-C^{14}
(Methyl-C^{14} Iodide)

METHOD I

$$C^*H_3OH \xrightarrow{HI} C^*H_3I$$

A. Murray III and A. R. Ronzio, J. Am. Chem. Soc., 74, 2408 (1952).

A. Procedure (Note 1)

The reactor (Figure IX, 2) consists of a thick-walled, Pyrex glass tube (20 × 250 mm.) with a thick-walled 10-ml. bulb, containing a glass-enclosed magnetic stirring bar at one end, and a pressure stopcock (Note 2) and connector to a vacuum manifold at the other. The greater part of the tube is jacketed for water cooling, and the stopcock bore is 4 mm., allowing charging of the tube through a long thin-stemmed funnel. To 7 ml. of hydriodic acid (d. 1.7), frozen in liquid nitrogen, is added 2.8–5.1 mmoles of methanol-C^{14} by vacuum distillation. The reactor is closed, and the stirred mixture is heated under reflux at 80–85° for 2 hours (Note 3), then cooled to room temperature. The product is distilled slowly through a purifying train, containing soda-lime and phosphorus pentoxide-on-glass wool (Note 4), into an evacuated flask, fitted with a pressure microstopcock. The transfer is completed by warming the reactor with hot water and cooling the receiver in a Dry Ice-bath; any noncondensable gas is pumped off. The yield of colorless product is 96.7–99.3% (Note 5).

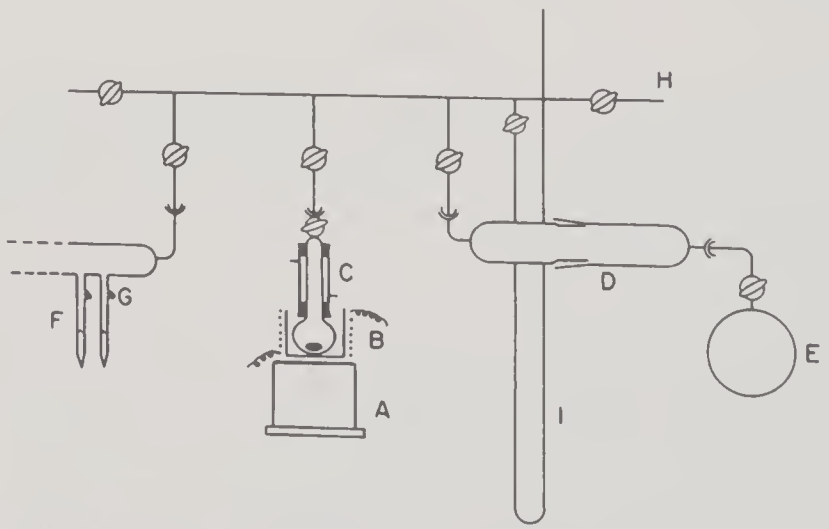


Fig. IX, 2. Apparatus for preparation of methyl halide (W. W. Foreman, A. Murray III and A. R. Ronzio). A, magnetic stirrer; B, electrically heated oil bath; C, water-jacketed, heavy-walled reaction tube; D, drying tube containing soda lime and phosphorus pentoxide-on-glass wool; E, gas-weighing flask with a pressure microstopcock; F, break-tubes containing methanol; G, steel ball bearing activated by a magnet; H, outlet to vacuum manifold; I, manometer.

METHOD II

$$C^*H_3OH \xrightarrow[H_2O]{I_2,P} C^*H_3I$$

B. M. Tolbert, J. Am. Chem. Soc., 69, 1529 (1947); M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 169; P. T. Adams, R. E. Selff and B. M. Tolbert, J. Am. Chem. Soc., 74, 2416 (1952).

A. Procedure

The apparatus consists of a vacuum line and a heavy-walled glass bomb tube, which is closed with an 8-mm. stopcock (secured by a clamp) and jacketed in the upper-half to permit refluxing (Note 6). To 10 g. of iodine, introduced through a long-stemmed funnel and cooled with liquid nitrogen, is added 2 g. of red phosphorus and 3 ml. of water, followed by the addition of 20–22.5 mmoles of crude methanol-C^{14} (see Method IV) by vacuum distillation. The tube is closed, and the mixture is refluxed for 1 hour on a steam-bath (Note 7). The crude product is vacuum-distilled, with pumping, into a trap cooled with liquid nitrogen (Note 8), then into a 100-ml. flask containing 10 ml. of water. The mixture is shaken for a minute at room temperature; then the product and some water are vacuum-distilled into a flask containing 3–4 g. of phosphorus pentoxide. The product is dried by shaking the mixture at room temperature for 30 minutes, then is distilled into a storage flask; yield 95% based on methanol (Note 9).

B. Notes

1. The procedure is a modification of the method of Norris,[1] and the apparatus is a modification of that described by Tolbert (see Method II).

2. The stopcock is lubricated with silicone grease, or Harshaw Stopcock Lubricant.

3. Heating periods as long as 6 hours have been used, but 2 hours is sufficient.

4. The soda lime (8–12 mesh) is previously dehydrated under high vacuum by heating with a burner. A convenient gas-drying tube is prepared by loosely packing a tube with pledgets of glass wool, swirled briefly in phosphorus pentoxide.

5. The purity has been checked by gravimetric iodine analysis of a trial preparation, by digestion with alcoholic silver nitrate for several days at 40°. The yield of silver iodide was 97.0% based on methanol, 100.3% based on methyl iodide.

Wagner[2] has reported that a sample of methyl-C^{14} iodide containing 1 mc./mmole undergoes decomposition by self radiolysis at the rate of 0.304% per year. The radiolysis of liquid methyl iodide has been studied extensively by Schuler and co-workers.[3]

6. Several diagrams of the vacuum line and suitable reaction and storage flasks are presented by Tolbert; also see methanol, Method IV.

7. The water considerably reduces the unknown pressure during heating by dissolving the hydrogen iodide formed. A cooling bath may be necessary to control the initial reaction.

8. The product is accompanied by some water, hydriodic acid and phosphine.

9. The phosphorus pentoxide reacts with most of the phosphine, and the dried product (n_D^{22} 1.5280) is suitable for Grignard reactions. Mahler,[4] however, has reported that the traces of phosphorus-containing impurities make it unsuitable for the preparation of D-glucose-1-C^{14}. Comparable yields have been obtained at the 15-mmole level.

C. Other Preparations

The large scale preparation of methyl-C^{14} iodide, by refluxing methanol-C^{14} with hydriodic acid, has been reported by Sowden[5] (83% yield) and Roberts[6] (93% yield b.p. 40.5–41° at 740 mm.). Melville[7] has obtained a yield of 83% (as the solid tetramethylammonium iodide) based on 0.326 g. of potassium formate (see methanol-C^{14}, Method III).

The preparation of methyl-C^{14} iodide by a modification of Method I has been reported by Cox[8] in detail (93% yield at the 5-mmole level), by Swan[9] and by Mahler[4] (90% yield, 95% maximum). Regier[10] has reported an estimated yield of 88%, at the 123-mmole level, by a procedure with an involved time-heating schedule.

Methyl-C^{11} iodide has been prepared by Harman[11] by passing methanol-C^{11} through a Zeisel apparatus.

Methyl-C^{14}-H_3^2 iodide has been prepared[12] by passing methanol-C^{14}-1-H_3^2 vapor through a refluxing solution of hydrogen-H^2 iodide made from phosphorus, iodine and water-H_2^2.

Methyl-C^{14} iodide has been prepared by Arnstein,[13] by Method II, and assayed as *S*-methyl-C^{14}-thiuronium picrate, m.p. 224–225° from acetone. Müller[14] has reported an 81.5% yield at the 23-mmole level.

[1]J. F. Norris, Am. Chem. J., *38*, 627 (1907).

[2]*C. D. Wagner* and V. P. Guinn, J. Am. Chem. Soc., *75*, 4861 (1953); R. M. Lemmon, Nucleonics, *11* (10), 44 (1953).

[3]R. C. Petry and R. H. Schuler, J. Am. Chem. Soc., 75, 3796 (1953); R. H. Schuler and W. H. Hamill, J. Am. Chem. Soc., *74*, 6171 (1952).

[4]H. R. Mahler, Atomic Energy Commission Report, AECD-2400; Nuc. Sci. Abstracts, *2*, 73 (1949).

[5]J. C. Sowden, J. Biol. Chem., *180*, *55* (1949); Science, *109*, *229* (1949).
[6]J. D. Roberts, R. E. McMahon and J. S. Hine, J. Am. Chem. Soc., *72*, 4237 (1950).
[7]D. B. Melville, J. R. Rachele and E. B. Keller, J. Biol. Chem., *169*, 419 (1947).
[8]J. D. Cox, H. S. Turner and R. J. Warne, J. Chem. Soc., *1950*, 3167.
[9]G. A. Swan and D. Wright, J. Chem. Soc., *1954*, 381.
[10]R. B. Regier and R. W. Blue, J. Org. Chem., *14*, 505 (1949).
[11]D. Harman, T. D. Stewart and S. Rubin, J. Am. Chem. Soc., *64*, 2293 (1942).
[12]J. R. Rachele, E. J. Kuchinskas, F. H. Kratzer and V. du Vigneaud, J. Biol. Chem., *215*, 593 (1955).
[13]H. R. V. Arnstein and R. Bentley, J. Chem. Soc., *1951*, 3436.
[14]E. Müller and W. Rundel, Ber., *88*, 917 (1955).

CARBON-C^{14} TETRACHLORIDE

$$C^*O_2 + 4H_2 \xrightarrow{Ni\text{-}ThO_2} C^*H_4 + 2H_2O$$

$$C^*H_4 + 4Cl_2 \xrightarrow{Light} C^*Cl_4 + 4HCl$$

W. H. Beamer, J. Am. Chem. Soc., *70*, 3900 (1948).

A. Procedure

(a) *Methane-C^{14}*. The carbon-C^{14} dioxide from 1–2 mmoles of barium carbonate-C^{14} is condensed into a trap of a vacuum system (0.1 μ) with liquid nitrogen. Purified hydrogen (Note 1) is admitted to the system to a pressure about 100 mm. below atmospheric. The liquid nitrogen-bath is replaced with Dry Ice-acetone to trap water and liberate carbon-C^{14} dioxide. The gas mixture is circulated over a nickel-thorium oxide catalyst at 330° (Note 2) by the action of a Töepler pump, and the water formed is collected in a second Dry Ice-acetone trap. The fall of pressure in the system, indicated by a mercury manometer, shows the progress of the reduction. When reaction is complete (30 minutes), the gases are passed over copper oxide at 280° to oxidize carbon-C^{14} monoxide and excess hydrogen to carbon-C^{14} dioxide and water. The product is then töeplered into an evacuated receiver; yield 95–98% (Note 3).

(b) *Carbon-C^{14} Tetrachloride*. The methane-C^{14} is töeplered into an evacuated photo-chlorination flask (1100 cc.) which is irradiated with ultraviolet light (Note 4). The pressure is read on a capillary manometer and, at 5–10 minute intervals, chlorine is added in increments of about one-tenth of the theoretical amount (Note 5). Finally, chlorine is added in 25% excess, and the reaction is continued for 1 hour. The products

are vacuum-distilled into a trap by means of liquid nitrogen, the trap is then cooled with a Dry Ice-acetone bath, and the hydrogen chloride and recoverable carbon-C^{14} dioxide are distilled off into another trap with liquid nitrogen. The residual chlorine and chlorinated methanes-C^{14} are irradiated in the liquid phase (Note 6) for 30 minutes to complete the reaction; then the chlorine and hydrogen chloride are removed by distillation. The residual mixture of carbon-C^{14} tetrachloride and hexachloroethane-C_2^{14} (1.5%) is separated by vacuum distillation of the carbon-C^{14} tetrachloride at 0° with Dry Ice-acetone cooling. The yield is 92–94%, and the purity is greater than 99% (Note 7).

B. Notes

1. Hydrogen is passed over platinized asbestos at 300°, soda lime and magnesium perchlorate.

2. The catalyst[1] is prepared by adsorbing nickel nitrate and thorium nitrate (20:1 ratio) on 8–16 mesh Silocel (a diatomaceous earth), roasting to the oxide and reducing with hydrogen at 330°.

3. The methane contains 2–6% hydrogen and 1–4% unreacted, but recoverable, carbon-C^{14} dioxide.

4. G. E. Type H-1 mercury sun lamp may be used.

5. The incremental addition of reagent prevents the formation of explosive mixtures of methane and chlorine and minimizes the formation of hexachloroethane. The yield of chlorinated ethanes and ethylenes increases with the radiation intensity.

6. The last mentioned trap is kept at −40° to prevent the chlorine pressure from exceeding atmospheric.

7. Infrared analysis showed less than 0.1% each of: methylene chloride, chloroform, phosgene and hexachloroethane. A sample of isotopic carbon tetrachloride was quantitatively converted back to barium carbonate-C^{14} with quantitative recovery of the initial activity; also see Beamer and Atchison[2] for a diagram of apparatus.

The distillation of carbon tetrachloride leads to an enrichment of C^{13} in the distillate.[4]

C. Other Preparations

Methane-C^{13} has been prepared in 86% yield[3] by acid hydrolysis of the Grignard reagent.

[1]R. N. Pease and P. R. Cheseboro, J. Am. Chem. Soc., *50*, 1466 (1928).
[2]W. H. Beamer and G. J. Atchison, Anal. Chem., *22*, 303 (1950).
[3]C. D. Wagner, D. P. Stevenson and J. W. Otvos, J. Am. Chem. Soc., *72*, 5786 (1950).
[4]P. Baertschi, W. Kuhn and H. Kuhn, Nature, *171*, 1018 (1953).

CHLOROETHANE-$C^{14}_{1/2}$

$$C^*H_3CH_2OH \xrightarrow{PCl_5} C^*H_3CH_2Cl \xrightarrow[AlCl_3]{} C^*H_3C^*H_2Cl$$

(a) (b)

R. M. Roberts, G. A. Ropp and O. K. Neville, J. Am. Chem. Soc., 77, 1764 (1955).

A. Procedure (Note 1)

(a) *1-Chloroethane-2-C^{14}*, (*Ethyl-2-C^{14} Chloride*). To 50 g. of phosphorus pentachloride at −198° is added 10 g. of ethanol-2-C^{14} by vacuum transfer. The mixture is warmed very slowly with stirring, the reaction never being allowed to become violent, while the evolved gases are passed through traps containing potassium hydroxide pellets and water, respectively. The system is scavanged by the expansion of several 10-ml. portions of carrier ethyl chloride, and the condensed product is redistilled; yield 61.2 g., b.p. 12° (742 mm.), radiochemical yield 65.4%.

(b) *Chloroethane-$C^{14}_{1/2}$*, (*Ethyl-$C^{14}_{1/2}$ Chloride*). To 2 g. of anhydrous aluminum chloride is added 20 g. of ethyl-2-C^{14} chloride. The mixture is quickly adjusted to room temperature and atmospheric pressure and is stored for 1 hour. The product is vacuum-distilled through a trap containing potassium hydroxide pellets, and redistilled several times at atmospheric pressure (Note 2), then is passed through bromine water, dried over Drierite, condensed and redistilled; yield 9.3 g., b.p. 12° (743 mm.). Degradation studies (Note 3) indicate that 41.8% of the initial activity is located at C-2, 83.6% rearrangement.

B. Notes

1. These preparations are incidental to a tracer study of the action of isomerization catalysts (Lewis Acids) on alkylaromatic compounds; see ethyl-2-C^{14}-benzene, (Note 1). Also see 2-chloro-2-methylbutane-1,4-$C^{14}_{1/2}$ and 2-bromo-2-methylbutane-1,2,3,4-$C^{14}_{1/4}$.

2. The product is condensed at −80° to remove ethylene. The instantaneous nature of the aluminum chloride-catalyzed condensation of ethylene with benzene has been pointed out by Francis.[1]

3. See ethyl-$C^{14}_{1/2}$-benzene. The observed reaction may be interpreted in terms of either displacement[2] or carbonium ion mechanisms.

[1] A. W. Francis and E. E. Reid, Ind. Eng. Chem., 38, 1194 (1946).

[2] H. C. Brown and M. Grayson, J. Am. Chem. Soc., 75, 6285 (1953).

IODOETHANE-C_2^{14}
(Ethyl-C_2^{14} Iodide)

$$C^*H_2 = C^*H_2 \xrightarrow{HI} C^*H_3C^*H_2I$$

W. J. Arrol and R. Glascock, J. Chem. Soc., *1949*, S335; Nature, *161*, 932 (1948).

A. Procedure

To 0.1 ml. of saturated hydriodic acid in a capillary tube equipped with a break-seal and frozen in a liquid nitrogen-bath is added 10 μmoles of ethylene-C_2^{14} by vacuum distillation (Note 1). The tube is sealed off with a torch and heated at 144° for 1 hour in a bath of boiling *o*-xylene. The tube is attached again to the vacuum line, the seal is broken, and the mixture is distilled into a flask containing 0.5 ml. of saturated sodium hydroxide solution. The flask is isolated from the system, and the mixture is boiled briefly, then cooled in a Dry Ice-bath, while the supernatant ethyl-C_2^{14} iodide and unreacted ethylene-C_2^{14} are distilled into a receiver cooled with liquid nitrogen. This boiling and low-temperature vacuum distillation process is repeated four times. The product is isolated from the distillate by distillation at the temperature of liquid oxygen (–183°) into a trap cooled with liquid nitrogen and connected to a McLeod gauge. The product is dried for 5 minutes with 0.1 g. of anhydrous magnesium perchlorate, then measured as vapor in the McLeod gauge (Note 2); yield 90–95% (Note 3).

B. Notes

1. The tube has a capacity such that the ethylene exerts a pressure of about 0.5 atmospheres at room temperature. Several diagrams of the apparatus are presented by Arrol. The main vacuum system may be protected from acid vapor by evacuating through an ancillary guard tube packed with calcium chips.

2. Ethyl iodide vapor may be manipulated with greased stopcocks, but the rate of loss into the grease is initially 5% per hour. Storage for any length of time is done best in a trap sealed with mercury.

3. Arrol has described the preparation of ethylmagnesium-C_2^{14} iodide at the micromole level by reaction in ethereal solution with a magnesium mirror in a small tube. The yield of Grignard reagent, determined by measuring the volume of ethane-C_2^{14} produced upon hydrolysis, is less than 55%.

C. Other Preparations

Ethyl-C_2^{13} iodide has been prepared by Wood,[1] by passing ethanol-C_2^{13}, in a stream of nitrogen, through boiling 57% hydriodic acid and a bead tower containing 20% cadmium chloride and 20% barium chloride solution.

The preparation of ethyl-C_2^{14} iodide by the described procedure has been reported by Kilner.[2]

The preparation of chloroethylene-C_2^{14}, (vinyl-C_2^{14}chloride), by a smiilar method has been reported by Beamer.[3] Acetylene-C_2^{14} was reacted at 120° with dry hydrogen chloride over charcoal impregnated with mercuric chloride catalyst. The product was frozen out from the recycled acetylene-C_2^{14}, and reaction time was kept short to prevent a second addition to the olefin. Poly(vinyl-C_2^{14}) chloride was formed by catalytic polymerization at 50° in a sealed capillary, see C^{14}-polyvinyl chloride.

[1]H. G. Wood, J. Biol. Chem., *194*, 905 (1952).

[2]A.E.H. Kilner, H. S Turner and R. J. Warne, *Isotope Conference, 1954*, Vol. II, Academic Press, New York, 1954, p. 23.

[3]W. H. Beamer, Nucleonics, 7 (3), 55 (1950).

1-BROMOPROPANE-1-C^{14}

$$CH_3CH_2C^*OOH \xrightarrow{MOH} CH_3CH_2C^*OOM \xrightarrow[CuCr_2O_4]{H_2} CH_3CH_2C^*H_2OH \xrightarrow{PBr_3}$$

(a)

$\downarrow I_2, P$

$CH_3CH_2C^*H_2Br$ (b) $\qquad$ $CH_3CH_2C^*H_2I$ (c)

P. T. Adams, R. E. Selff and B. M. Tolbert, J. Am. Chem. Soc., 74, 2416 (1952).

A. Procedure

(a) *1-Propanol-1-C^{14}* (Note 1). A solution of 1.44 g. (15 mmoles) of sodium propionate-1-C^{14} in 5 ml. of water is treated with a cation exchange resin to form the free acid (Note 2). The filtered solution is added to a slurry of 90% cadmium and 10% nickel hydroxides, prepared from 1.70 g. (7.43 mmoles) of cadmium chloride $\cdot 2\frac{1}{2} H_2O$ and 0.24 g. (0.83 mmole) of nickel nitrate hexahydrate (Note 3). The salt solution is evaporated to dryness (Note 4); the residue is mixed with 1.5 g. of copper chromite catalyst in a small stainless steel reaction vessel (Note 5), and all moisture is removed by evacuation for 4–8 hours at 50 μ. The reactor is filled with electrolytic hydrogen to a pressure of

235 atmospheres and shaken at 240° for 9 hours. The reactor is allowed to cool, excess hydrogen is released through a spiral trap at −196° (containing a fritted-disc), and the product is vacuum-distilled into the cold trap by warming the reactor to 80° for 5 hours. The yield is 1.14 g. of a 74% propanol solution in water, 94% (Note 6).

(b) *1-Bromopropane-1-C^{14}, (Propyl-1-C^{14} Bromide)* (Note 7). The mixture of water and 1-propanol-1-C^{14} is vacuum-distilled into a bromination flask, which is then fitted with an addition funnel and low-temperature condenser. Phosphorus tribromide (4 ml.) is added dropwise, and the mixture is refluxed for 3 hours. The product is washed with water and dried over phosphorus pentoxide; yield 1.26 g., 73% based on propanol.

(c) *1-Iodopropane-1-C^{14}, (Propyl-1-C^{14} Iodide)* (Note 8). The mixture of water and 1-propanol-1-C^{14} is vacuum-distilled into a heavy-walled glass bomb tube (Note 9) containing 10 g. of iodine, 0.62 g. of red phosphorus and 3 ml. of water. The tube is closed with a large-bore pressure stopcock, and the mixture is refluxed for 2 hours. The product is vacuum-distilled into a trap where it is washed with water and dried over phosphorus pentoxide; yield 86% based on propanol.

B. Notes

1. This general method, developed from the method of Richardson and Taylor[1] (see methanol-C^{14}, Method II), has been applied to straight and branched chain fatty acids through C_5 at the 20-mmole level. The product is not contaminated by the alcohols that arise from scission of the solvent ethers commonly employed in reductions with lithium aluminum hydride (see methanol-C^{14}, Method I). For other isotopic procedures, see Fries and Calvin (1-propanol-1-C^{14}) and Gordon and Heimel (1-propanol-1-C^{13}). Also see 2-methyl-1-propanol-1-C^{14}.

2. A suitable resin is Dowex 50 (in the acid-form), 250–300 mesh, 15 ml.

3. A solution of the salts in 10 ml. of water is treated with excess sodium hydroxide; then the precipitate is centrifuged and washed three or four times with water.

4. The conversion of sodium propionate-1-C^{14} to cadmium-nickel propionate-1-C^{14} is quantitative.

5. Micro Series Reaction Vessel (115 ml.), American Instrument Co., Silver Springs, Md. In preparing the catalyst,[2] the final acid wash is with 5% nitric acid, instead of acetic acid, to eliminate possible product contamination with ethanol. The copper chromite catalyst reduces the intermediate ester to alcohol.[3] A catalyst prepared under slightly different conditions has been reported to form chiefly hydrocarbons; see pentane-1-C^{14} and pentane-3-C^{14}.

6. The product was converted to barium carbonate with a molar specific activity of 95.6% of the theoretical value. Under other conditions of time, temperature and pressure, the hydrogenation product is chiefly hydrocarbon.

7. The procedure is that described by Tolbert;[4] also see Fries and Calvin (propyl-1-C^{14} bromide). The phosphorus tribromide method is superior to the hydrobromic-sulfuric acid method for preparing *sec.*-butyl, propyl, isopropyl and isobutyl bromides.[5]

8. The method is that described by Tolbert (methyl-C^{14} iodide, Method II); also see Gordon and Heimel (propyl-1-C^{13} iodide).

9. The upper-half of the tube is jacketed and water-cooled.

10. Formate-C^{14} may be prepared from carbon-C^{14} dioxide (85–90% yield) by the method of Melville, Rachele and Keller (see sodium formate-C^{14}, Method II).

11. The yield varies from 70–90%. The conversion of methanol is 95%, or better; see methanol-C^{14} (Method II) and methyl-C^{14} iodide.

C. Other Preparations

1-Bromo-2-methylpropane-1-C^{14} has been prepared by Coon[6] in 52% yield from 2-methylpropionic-1-C^{14} acid, by reduction with lithium aluminum hydride, followed by treatment of the 2-methyl-1-propanol-1-C^{14} with phosphorus tribromide.[5]

2-Iodopropane-1,3-C_2^{14} has been prepared by Ostwald[7] (75% yield based on barium carbonate) by hydrogenation of 2-propanone-1,3-C_2^{14} over copper chromite, followed by treatment of the 2-propanol-1,3-C_2^{14} with phosphorus and iodine.

By the same procedure, the following series of compounds has been prepared from the corresponding carboxylic acid, in the indicated yields. The alcohol yield is calculated on the basis of experimental efficiency of converting the alcohol to the halide. The yield of these alcohols might be improved by determining the optimum conditions of time, temperature, pressure and salt composition.

Methanol-C^{14} (85–90%, Note 10); iodomethane-C^{14}, (methyl-C^{14} iodide) (80%) (Note 11).

Ethanol-1-C^{14} (95%), iodoethane-1-C^{14}, (ethyl-1-C^{14} iodide) (85%); see C_1^{14}-ethanol, Method I.

1-Butanol-1-C^{14} (75%), 1-bromobutane-1-C^{14}, (butyl-1-C^{14} bromide) (54%).

2-Methyl-1-propanol-1-C^{14}, (isobutyl-1-C^{14} alcohol), (80%); 1-iodo-2-methylpropane-1-C^{14}, (isobutyl-1-C^{14} iodide) (65%).

1-Pentanol-1-C^{14}, (pentyl-1-C^{14} alcohol), (80%), 1-bromopentane-1-C^{14}, (pentyl-1-C^{14} bromide) (57%).

3-Methyl-1-butanol-1-C^{14}, (isopentyl-1-C^{14} alcohol), (70%); 1-bromo-3-methylbutane-1-C^{14}, (isopentyl-1-C^{14} bromide) (50%).

The preparation of ethyl-1-C^{14} iodide by reaction of ethanol-1-C^{14} with constant-boiling hydriodic acid has been reported by Finkelstein.[8]

[1]A. S. Richardson and J. E. Taylor, U. S. 2,340,344; 2,340,687 to 2,340,691; Chem. Abstracts, *38*, 3990, 4269 (1944).

[2]H. Adkins, *Reactions of Hydrogen with Organic Compounds over Copper-Chromium Oxide and Nickel Catalyst*, Univ. of Wisconsin Press, Madison, 1937, p. 12; *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 144.

[3]K. Folkers and H. Adkins, J. Am. Chem. Soc., *54*, 1145 (1932).

[4]B. M. Tolbert, F. C. Christenson, F. N. H. Chang and P. P. T. Sah, J. Org. Chem., *14*, 525 (1949).

[5]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 358; Coll. Vol. I, 1941, p. 40.

[6]M. J. Coon and S. Gurin, J. Biol. Chem., *180*, 1159 (1949).

[7]R. Ostwald, Atomic Energy Commission Report, UCRL-2154; Nuc. Sci. Abstracts, 7, 3404 (1953).

[8]A. Finkelstein and A. W. Noyes, Jr., Discussions Faraday Soc., *14*, 81 (1953).

C^{14}-HALOPROPENES
(C^{14}-Allyl Halides)

$$CH_2{=}CHC^*H_2OH \xrightarrow[C_5H_5N]{PBr_3} C^*H_2{=}CHC^*H_2Br$$

R. F. Nystrom and J. C. Leak, J. Am. Chem. Soc., *75*, 3039 (1953).

A. Procedure (Note 1)

3-Bromopropene-1,3-$C^{14}_{1/2}$, (Allyl-1,3-$C^{14}_{1/2}$ Bromide). The halide is prepared from allyl-1-C^{14} alcohol and phosphorus tribromide by a modification of the method of Young and Lane,[1] which follows. Degradation studies show that 46% of the isotope is rearranged to C-3 (Note 2).

The carbinol (0.3 mole) and 8.0 ml. of dry pyridine are mixed with 15 g. of powdered Dry Ice in a 100-ml. distilling flask (equipped with a dropping funnel and a drying tube attached to the side arm) cooled with a Dry Ice-alcohol bath. Phosphorus tribromide (11.5 ml.) is added dropwise with shaking over a period of 1.5 hours. The mixture is warmed slowly to $-25°$, to remove Dry Ice, then distilled at 3 mm. (Note 3). The distillate is washed with a mixture of crushed ice and dilute sodium bicarbonate, followed by ice water, then is dried over calcium chloride at $-15°$ and redistilled.

Allyl-1-C^{14} bromide is formed without rearrangement by treating an acetone solution of allyl-1-C^{14} tosylate with sodium bromide.

Allyl-1,3-$C^{14}_{1/2}$ chloride is prepared from allyl-1-C^{14} alcohol and thionyl chloride by the method of Meisenheimer and Link.[2] Degradation studies (Note 2) show that 51% of the isotope is rearranged to C-3.

B. Notes

1. These preparations were designed to determine the extent of allylic rearrangement when the halide is prepared from allyl alcohol by several methods; see the study by Young and Lane[1], which avoids thermal rearrangement into an equilibrium mixture.

2. Both the starting material and the bromide are degraded with ozone, and the formaldehyde-C^{14} is assayed.

3. Allyl bromide boils at 71° under atmospheric pressure.

[1]W. G. Young and J. F. Lane, J. Am. Chem. Soc., *59*, 2051 (1937).

[2]J. Meisenheimer and J. Link, Ann., *479*, 211 (1930).

HEXACHLOROPROPENE-1,3-$C^{14}_{1/2}$

$$C_6H_5COC^*H_3 \xrightarrow{Cl_2} C_6H_5COC^*Cl_3 \xrightarrow{NaOH} C^*HCl_3 \xrightarrow[AlCl_3]{Cl_2C{=}CCl_2}$$

(a)

$$Cl_2C^*HCCl_2CCl_3 \xrightarrow[CH_3OH]{NaOH} Cl_2C^* {=} CClC^*Cl_3 \xrightarrow[H_2SO_4]{O_3} C^*O_2 + C^*Cl_3COOH$$

(b)

H_2SO_4 ↙ ↓ SbF_3, $SbCl_5$

$Cl_2C^* {=} CClC^*OOH$ (d)

$Cl_2C^* {=} CClC^*F_3$ (c)

C. H. Shuford, Jr., D. L. West and H. W. Davis, J. Am. Chem. Soc., 76, 5803 (1954); H. W. Davis, Atomic Energy Commission Report, ORO-122; Nuc. Sci. Abstracts, *8*, 4025 (1954).

A. Procedure

(a) *Chloroform-C^{14}* (Note 1). The apparatus consists of a 500-ml., three-necked flask, equipped with a stirrer, thermometer, reflux condenser and chlorine dispenser. Chlorine is bubbled into a stirred solution of 24.0 g. (200 mmoles) of acetophenone-2-C^{14} in 100 ml. of glacial acetic acid. After the temperature rises to a peak of 70–80° (during 30–40 minutes) and begins to fall, the chlorine supply is turned off, and the cooled solution is treated with 50 g. (0.6 mole) of fused sodium acetate. Chlorine is bubbled through the clear solution, and the temperature is raised to 110–115° until a permanent yellow color is produced. The crude 2,2,2-trichloracetophenone-2-C^{14} is washed with five portions of water in the reaction flask, then treated with 20 g. (0.5 mole) of sodium hydroxide in 50 ml. of water. The condenser is set for steam distillation,

and the stirred mixture is warmed to 55–60° until the exothermic reaction commences, distillation being continued until the vapor reaches 95°. The product is dried over calcium chloride and distilled from anhydrous aluminum chloride; yield 16.4 g., 68.5% (Note 2).

(b) *Hexachloropropene-1,3-$C^{14}_{1/2}$* (Note 3). A mixture of the chloroform-C^{14} (137 mmoles), 10 g. of powdered anhydrous aluminum chloride and 21.6 g. (130 mmoles) of tetrachloroethylene (distilled from $AlCl_3$) is heated under reflux. The mixture begins to boil at a liquid temperature of about 65°, and the temperature rises slowly to about 105° in 10–15 minutes, as the mixture exhibits a series of color changes. The cooled mixture is poured into ice water and washed with several portions of water. A solution of 5.2 g. (130 mmoles) of sodium hydroxide in 32 ml. of methanol is added slowly with stirring to the colorless 1,1,1,2,2,3,3-heptachloropropane-3-C^{14} (Note 4), while the temperature is maintained at 30–35°. The mixture is diluted with 200 ml. of water, and the dehydrohalogenation product is washed with water, dried over calcium chloride and fractionated; yield 23.6 g. (47.5% based on acetophenone), b.p. 120° (49 mm.) (Note 5). The molar specific activity is the same as that of the starting material.

Degradation studies (Note 7) indicate that 75% of the initial activity is located at C-1, with 8% in the rest of the molecule and the balance unaccounted for. Infrared analysis shows the presence of some oxygen containing contaminant which is removed effectively by passage with methanol through a silica gel column (Note 6). However, this treatment, as well as purification by distillation (4–5 hours at 120°), results in isomerization of the molecule, 41–44% of the initial activity being found at C-1 and 44–48% in the rest of the molecule (Note 8).

(c) *1,1,2-Trichloro-3,3,3-trifluoropropene-1,3-$C^{14}_{1/2}$* (Note 9). A mixture of 32.5 g. (125 mmoles) of crushed antimony trifluoride and 2 ml. of antimony pentachloride is heated in a Claisen flask until some sublimation is observed. Heating is reduced and 16.0 g. (64 mmoles) of hexachloropropene-1,3-$C^{14}_{1/2}$ is added dropwise; the product is distilled at 90–95° and collected under concentrated hydrochloric acid. The reaction mixture finally is heated until the vapor temperature is 125°. The distillate is washed with dilute hydrochloric acid, dilute sodium hydroxide and water, then dried over calcium chloride and distilled through a 3-foot Vigreux column (10 mm. diameter); yield 5.2 g. (41%), b.p. 85–86°. Degradation studies indicate that 42–44% of the activity of the acetophenone-2-C^{14} is located at C-1 (Notes 7 and 8).

(d) *Trichloroacrylic-1,3-$C^{14}_{1/2}$ Acid.* A mixture of 1 ml. of hexachloropropene-1,3-$C^{14}_{1/2}$ and 1.5 ml. of concentrated sulfuric acid is heated with vigorous shaking until solution has taken place. After being poured into 70 ml. of water, the solution is extracted with two 10-ml. portions of chloroform. Solvent removal and recrystallization of the residue from

carbon disulfide yield the product, m.p. 76–77°, having the same molar specific activity as the starting material.

B. Notes

1. The procedure is a modification of the methods of Aston[1] and Boyer.[2]

2. The yield was 78% at the 340-mmole level.

3. The product is partially isomerized with regard to C^{14}. The procedure for the condensation step is adapted from the method of Prins,[3] who reported an 85% yield of 1,1,1,2,2,3,3-heptachloropropane, m.p. 29.4°, for the reversible reaction. At this scale the short reaction time is better than that recommended by Farlow.[4]

4. 1,1,1,2,2,3,3-Heptachloropropane boils at 110–113° (10 mm.), m.p. 29–30°.[4]

5. The yield is 51.5% based on chloroform at the 264-mmole level; b.p. 120–121° (50 mm.). A sample purified by chromatography on silica gel gave n_D^{24} 1.5478.

6. As hexachloropropene cannot be satisfactorily assayed by wet combustion, it may be converted to trichloroacrylic acid (d) for this purpose.

7. Directions are given by Davis for cleaving the double bond by passing ozonized oxygen[5] through a solution of the compound in carbon tetrachloride for 4 hours. The ozonide solution is decomposed by dropping into 5% sulfuric acid solution, and the evolved carbon-C^{14} dioxide is swept with nitrogen into a barium hydroxide solution. The acid solution is basified with sodium hydroxide, washed with carbon tetrachloride, acidified and extracted with ether. The extract of trichloroacetic-2-C^{14} acid is concentrated, and the residue is converted to the *S*-benzylthiuronium salt for assay.

8. This allylic rearrangement[6] of hexachloropropene, which was undetectable without the assymetry produced by the carbon isotope, makes it impossible to draw any conclusions regarding the mechanism of allylic fluorination (c).

9. The fluorination method is essentially that described by Whaley.[6]

C. Other Preparations

A preparation of chloroform-C^{14} from acetaldehyde-C_2^{14} is described in detail by Reitz.[7]

A mixture of 100 g. of high-test calcium hypochlorite and 0.5 g. of sodium hydroxide is ground with 250 ml. of water. To the resulting solution, decanted into a Claisen flask, is added 8.14 g. (183.6 mmoles) of acetaldehyde-C_2^{14} with a little water. Reaction is immediate with considerable frothing. The fraction distilling at 55–63° is collected in

a separatory funnel cooled with an ice-bath, and the product layer is separated; yield 59%, n_D^{20} 1.4452. The distillation residue contains calcium formate-C^{14}.

The distillation of chloroform-C^{13} leads to an enrichment in the distillate, the difference in boiling temperatures being 0.02°.[8]

[1]J. G. Aston, J. D. Newkirk, J. Dorsky and D. M. Jenkins, J. Am. Chem. Soc., *64*, 1413 (1942).
[2]W. M. Boyer, R. B. Bernstein, T. L. Brown, and V. H. Dibeler, J. Am. Chem. Soc., *73*, 770 (1951).
[3]H. J. Prins, Rec. trav. chim., *54*, 249 (1935).
[4]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 312.
[5]*Organic Syntheses*, Vol. 26, Wiley, New York, 1946, p. 63.
[6]A. M. Whaley and H. W. Davis, J. Am. Chem. Soc., *70*, 1026 (1948).
[7]H. C. Reitz, F. J. Carleton and J. E. Christian, J. Am. Pharm. Assoc., Sci. Ed., *42*, 85 (1953).
[8]P. Baertschi, W. Kuhn and H. Kuhn, Nature, *171*, 1018 (1953).

3-CHLORO-2-METHYLPROPENE-3-C^{14}

$$(CH_3)_2CHC^*OOH \xrightarrow[H_2SO_4]{(CH_3)_2CHCH_2OH} (CH_3)_2CHC^*OOCH_2CH(CH_3)_2 \xrightarrow[CuCr_2O_4]{H_2}$$
(a)

$$(CH_3)_2CHC^*H_2OH \xrightarrow{P + I_2} (CH_3)_2CHC^*H_2I \xrightarrow{KOH} (CH_3)_2C{=}C^*H_2 \xrightarrow[0°]{Cl_2}$$
(b) (c) (d)

$$CH_2{=}\underset{\displaystyle CH_3}{\underset{|}{C}}C^*H_2Cl \xrightarrow[2.\ Zn]{1.\ O_3} HCHO + CH_3COC^*H_3$$
(e)

W. Reeve, D. H. Chambers and C. S. Prickett, J. Am. Chem. Soc., *74*, 5369 (1952).

A. Procedure

(a) *Isobutyl 2-Methylpropionate-1-C^{14}*. To a mixture of 11.5 g. of isobutyl alcohol, 40 ml. of dry benzene and the sodium 2-methylpropionate-1-C^{14} prepared from 144 mmoles of isopropyl bromide is added dropwise 10 ml. of sulfuric acid. The mixture is refluxed for 4 hours, the water formed being collected in a moisture trap.[1] The benzene solution is washed with dilute sodium carbonate solution and saturated sodium chloride, then dried over calcium chloride. The solution is refluxed for 4 hours over Raney nickel (Note 1) and fractionally distilled through a Widmer column; yield 12.7 g. (62% based on the Grignard reagent), b.p. 144–147°.

(b) *2-Methyl-1-propanol-1-C^{14}* (Note 2). The isobutyl 2-methylpropionate-1-C^{14} (12.7 g.) is dissolved in 15 ml. of alcohol and hydrogenated over 6 g. of copper chromite catalyst for 6 hours at 250°; the initial

pressure at room temperature is 3500 p.s.i. The mixture is dissolved in ether and washed with 30% calcium chloride solution, then dried over potassium carbonate and fractionally distilled through a Widmer column. Low boiling fractions are mixed with 8.4 g. of carrier and refractionated, making the total yield 16.7 g. (63% corrected), b.p. 104–107°.

(c) *1-Iodo-2-methylpropane-1-C^{14}* (Note 2). To a mixture of 15.9 g. (214 mmoles) of 2-methyl-1-propanol-1-C^{14} and 2.4 g. (78 mg. atoms) of red phosphorus is added 27.2 g. (214 mg. atoms) of iodine in two portions with shaking and cooling. The mixture is stored overnight at room temperature, then treated with 0.75 g. (24 mg. atoms) of white phosphorus, with warming to complete the reaction of the phosphorus, and finally refluxed for 1 hour. The crude product is distilled into ice and water, the last of the product being steam-distilled after the addition of 30 ml. of water. To the crude colored product (21 ml.) is added 28 ml. of 95% alcohol and 4 ml. of concentrated ammonium hydroxide. The solution is stored overnight, then diluted with water, and the product is separated and distilled; yield 25.4 g. (64%), b.p. 116–120°.

(d) *2-Methylpropene-1-C^{14}*. A solution containing 21.9 g. (332 mmoles) of potassium hydroxide (85%), 85 ml. of absolute ethanol and 25.4 g. (138 mmoles) of 1-iodo-2-methylpropane-1-C^{14} is heated under reflux in an atmosphere of carbon dioxide. The evolved gas is passed through a series of water scrubbers, ice-cooled traps and a mercury valve, then collected over 5% sodium hydroxide in an inverted, calibrated 5-l. flask. The system is swept with carbon dioxide to complete the transfer of product; yield 117 mmoles (85%), 3110 ml. at 32° (753 mm.). Degradation studies indicate that there is no isotopic rearrangement (Note 3).

(e) *3-Chloro-2-methylpropene-3-C^{14}*, (*C_1^{14}-Methallyl Chloride*) (Note 4). 2-Methylpropene-1-C^{14} (77.5 mmoles) and about 100 mmoles of chlorine, each dried by passage through calcium chloride, are mixed and reacted in an 0.8-mm. Y-shaped capillary tube cooled with an ice-bath. The flow rate of the two gases is 156 and 204 ml./min., respectively. The product is collected in a 4.5 × 25-cm. tower filled with crushed ice and connected to a Mariotte flask (Note 5). The Y-tube is swept with carbon dioxide at the end of the run, and the organic phase is separated, washed with water and combined with two 2-ml. portions of butyl ether, used to extract the aqueous phase. The solution is dried over calcium chloride and fractionated through a modified Widmer column; yield 4.2 g. (60%), b.p. 72–74° (Note 6). Degradation studies (Note 7) indicate that the isotope is located at C-3 as a result of rearrangement.

B. Notes

1. This procedure removes catalyst poisons.
2. See propyl-1-C^{14} iodide.

3. Directions are given by Reeve for the ozonolysis[2] of the C_1^{14}-isobutylene, forming formaldehyde-C^{14} dimedon[3] (22% yield) and nonisotopic acetone 2,4-dinitrophenylhydrazone[4] (30% yield).

4. The method is essentially that of Burgin[5] and co-workers, who studied the reaction extensively. The procedure is incidental to a study of the reaction mechanism of low-temperature chlorination of isobutylene. The first step apparently involves the addition of a positive chlorine ion to the center of high electron density of the olefin, the terminal carbon atom. The second step, however, does not involve the addition of a negative chlorine; instead the chloro-*t*-butylcarbonium ion loses a proton from a methyl group to form the unsaturated monohalide with formation of a new double bond.

5. The flask serves as a pressure regulator and trap for unreacted isobutylene.

6. 1-Chloro-2-methylpropene, formed[5] to the extent of 2.5% under these conditions, boils at 68.1°.

7. Directions are given by Reeve for ozonolysis of the olefin and decomposition of the ozonide with platinized zinc dust in boiling water, converting the 1-chloro-2-propanone-1-C^{14} to 2-propanone-1-C^{14} 2,4-dinitrophenylhydrazone. The formaldehyde-C^{14} dimedon formed contains 3.7% of the activity of the starting material and is believed to be due to the formation of the by-product referred to in Note 6.

[1]*Organic Syntheses*, Coll. Vol. III, Wiley, New York, 1955, p. 382.
[2]J. M. Church, F. C. Whitmore and R. V. McGrew, J. Am. Chem. Soc., *56*, 176 (1934).
[3]E. C. Horning and M. G. Horning, J. Org. Chem., *11*, 95 (1946).
[4]N. R. Campbell, Analyst, *61*, 391 (1936).
[5]J. Burgin, W. Engs, H. P. A. Groll and G. Hearne, Ind. Eng. Chem., *31*, 1413 (1939).

1-IODOBUTANE-3-C^{14}
(Butyl-3-C^{14} Iodide)

$$CH_3C^*H_2MgI \xrightarrow{\text{ethylene oxide}} CH_3C^*H_2(CH_2)_2OMgI \xrightarrow{HI} CH_3C^*H_2(CH_2)_2I$$

I. Siegel and V. Lorber, J. Biol. Chem., *189*, 571 (1951).

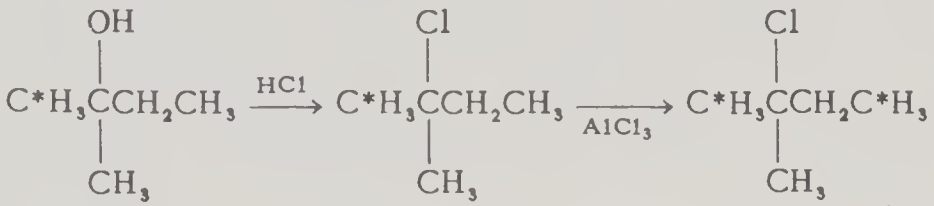
Procedure

The Grignard reagent prepared from ethyl-1-C^{14} iodide is reacted with ethylene oxide, according to a suitable adaptation of the method of Dreger;[1] then the reaction mixture is evaporated to dryness at reduced pressure. The ethyl ether remaining bonded to the butoxymagnesium-3-C^{14} iodide subsequently gives rise to nonisotopic ethyl iodide. The

residual Grignard complex is decomposed with concentrated hydriodic acid, and the product is isolated by ether extraction and purified by fractional distillation.

[1]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 306.

2-CHLORO-2-METHYLBUTANE-1,4-$C^{14}_{1/2}$



(a) (b)

J. D. Roberts, R. E. McMahon and J. S. Hine, J. Am. Chem. Soc., 72, 4237 (1950); 71, 1896 (1949).

A. Procedure

(a) *2-Chloro-2-methylbutane-1-*C^{14} (Note 1). 2-Methyl-2-butanol-1-C^{14} is converted to the chloride by distillation with concentrated hydrochloric acid. The organic portion of the distillate is washed with cold water and saturated salt solution, then dried over calcium sulfate and distilled through a 50-cm. glass helix-packed column (Note 2); yield 85–95%, b.p. 41–43° (180 mm.), n_D^{25} 1.4012. Degradation studies (Note 3) indicate that there is no isotopic rearrangement.

(b) *2-Chloro-2-methylbutane-1,4-*$C^{14}_{1/2}$. The apparatus consists of a 200-ml. three-necked flask equipped with a magnetic stirrer, solid addition tube, and a connection to a series of three Dry Ice-cooled traps. To 15 g. of 2-chloro-2-methylbutane-1-C^{14}, at 0° with stirring, is added 0.1 g. of anhydrous aluminum chloride. Reaction usually begins at once with evolution of hydrogen chloride and formation of a brownish lower layer of polymer. At 90 seconds the system is evacuated, and the volatile material is collected in the traps within about 2 minutes. The distillate is mixed with 10 ml. of dry xylene (distillation base) and 3 ml. of dimethylaniline, and fractionated through the packed column; yield 10.0 g. (67%), b.p. 39–40° (175 mm.), n_D^{25} 1.4012. Degradation studies (Note 3) indicate that 66.3% of the activity is found at C-1, 1.9% at C-3, and 31.8% at C-4 (Note 4).

B. Notes

1. The procedure is an adaptation of the method of Norris.[1]

2. Addition of a few ml. of dimethylaniline eliminates the evolution of hydrogen chloride,[2] a sign of decomposition, during distillation.

3. The degradative procedure is outlined under 2-bromo-2-methylbutane-$C^{14}_{1/4}$, Note 4.

4. The apparent activity at C-3 is considered illusory, and essentially complete isotopic equilibrium was established between C-1 and C-4.

C. Other Preparations

By the same procedure from 2-methyl-2-butanol-2-C^{14} is prepared 2-chloro-2-methylbutane-2-C^{14} and 2-chloro-2-methylbutane-2,3-$C^{14}_{1/2}$ (recovery 57%), having 63.7% of the activity at C-2 and 35.1% at C-3. The product is considered to be about 70% rearranged with respect to the statistically possible exchange between C-2 and C-3.

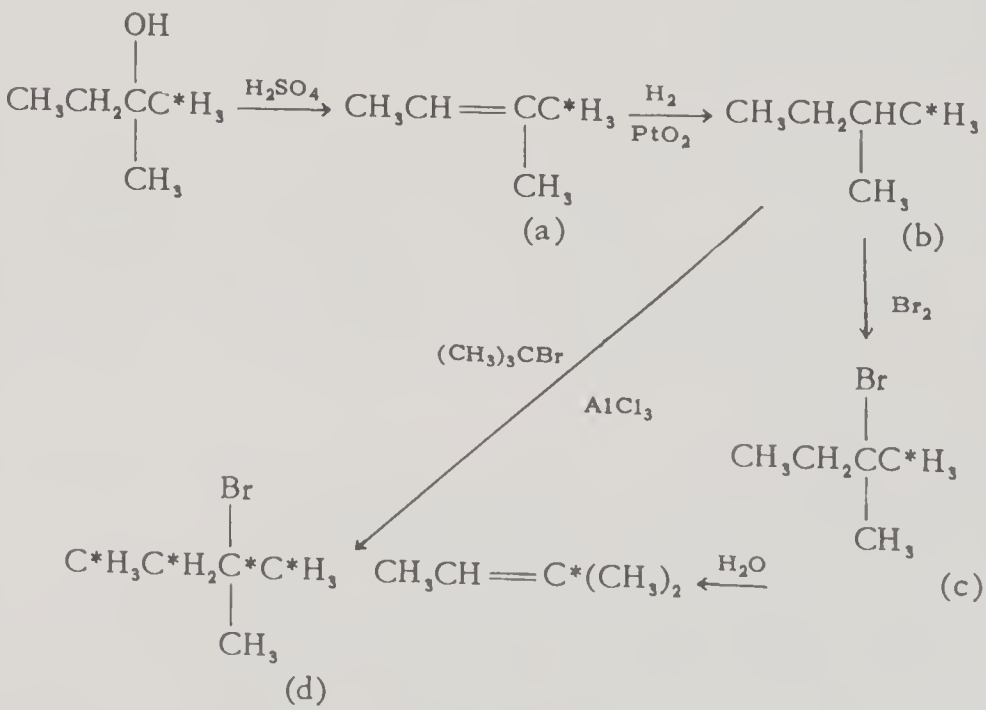
From 2-methyl-2-propanol-2-C^{14} is prepared 2-chloro-2-methylpropane-2-C^{14}; yield 73%, b.p. 50–50.5°, n_D^{25} 1.3818.

The aluminum chloride catalyzed rearrangement of 2-chloro-2-methylpropane-2-C^{14} takes place only under drastic conditions (47% recovery) and with only 1–3% of the activity rearranged to C-1.

[1]J. F. Norris, M. Watt and R. Thomas, J. Am. Chem. Soc., *38*, 1071 (1916); *Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 144.

[2]B. B. Corson, J. S. Thomas and D. D. Waugh, J. Am. Chem. Soc., *51*, 1950 (1929).

2-BROMO-2-METHYLBUTANE-$C^{14}_{1/4}$



J. D. Roberts and G. R. Coraor, J. Am. Chem. Soc., *74*, 3586 (1952).

A. Procedure

(a) *2-Methyl-2-butene-1-C^{14}*. A mixture of 15.3 g. of 2-methyl-2-butanol-1-C^{14} and 56 ml. of 18 *N* sulfuric acid is heated at 140° in a flask equipped with a 10-cm. indented column. The effluent gases are passed through a tower of potassium hydroxide pellets and condensed in a trap cooled with Dry Ice, the last of the product being swept into the trap with a stream of nitrogen; crude yield 8.6 g. (82%).

(b) *2-Methylbutane-1-C^{14}*. The olefin is dissolved in 25 ml. of acetic acid, in a flask equipped with a gas inlet tube and Dry Ice condenser, and hydrogenated at room temperature and atmospheric pressure for 9 hours over 0.8 g. of platinum oxide (Note 1). The product is distilled from the mixture under reduced pressure, bubbled through 50% potassium hydroxide solution and condensed in a trap cooled with Dry Ice. The product is dried by passage through a tower of potassium hydroxide, then fractionally redistilled through a 20-cm. column; yield 4.9 g. (39% based on the carbinol). The material used in isomerization studies is purified further by refluxing for 6 hours with alkaline sodium permanganate, drying and distilling.

(c) *2-Bromo-2-methylbutane-1-C^{14}*. The vapor-phase bromination of 8.0 g. of 2-methylbutane-1-C^{14} is performed in the illumination of two No. 2 Photoflood lamps at 20 cm. with the apparatus described by Roberts and Mazur[1] (Note 2). Bromine is carried into the reaction zone by a stream of dry nitrogen bubbling through bromine at room temperature. The yield is 10.7 g. (71%), b.p. 105–106° (Note 3). Degradation studies indicate that there is no isotopic rearrangement (Note 4).

(d) *2-Bromo-2-methylbutane-$C^{14}_{1/4}$*. The aluminum bromide-catalyzed halogen-hydrogen exchange between *t*-butyl halide and isopentane to yield *t*-amyl bromide[2] (Note 5) is performed in a flow apparatus[2] similar to one described by Swain and Kent.[3] Reaction takes place in a disc-shaped volume element located between two opposing jets, and radiating out a short distance into a quenching solution. The rates of flow and dimensions of the "cross fire" are such as to give a contact time of about 5 milliseconds.

A mixture of 7.0 g. (97 mmoles) of 2-methylbutane-1-C^{14} (the isopentane), 54 g. (360 mmoles) of *t*-butyl bromide in 15 ml. of olefin-free pentane, and a solution of 21 g. (80 mmoles) of aluminum bromide in 75 ml. of olefin-free pentane are forced under nitrogen pressure through opposed orifices into vigorously stirred ice-water. The organic phase is separated, washed with water, dried over magnesium sulfate and fractionally distilled; yield 9.0 g. (61%), b.p. 106–108°. Degradation studies (Note 5) indicate that the isotopic distribution (completely equilibrated) is 59 ± 2% at C-1, 30 ± 1% at C-4, 7 ± 3% at C-3, and 5 ± 3% at C-2 (Note 6).

B. Notes

1. A water-bath at room temperature prevents the solvent from freezing.

2. The apparatus consists of an illuminated, glass spiral with a gas inlet and connections to a reaction flask and to a low temperature reflux-condenser.

3. No isomeric monobromides are detected. Photochemical chlorination, however, gives only mixed monochlorides (90%), b.p. 91–96.5 °.

4. Degradation[4] is accomplished by dehydrohalogenation[5] with boiling water, forming trimethylethylene (b.p. 36–36.5 °), which then is degraded by the procedure described under 2-methyl-2,3-butanediol-2-C^{14}. Dehydrohalogenation with sodium ethoxide forms considerable 2-methyl-1-butene.

5. Halogen-hydrogen exchange takes place within 1 millisecond between isoparaffins[2] and tertiary or secondary alkyl halides in the presence of aluminum bromide. As the contact time increases, further isomerization takes place with partial shift of the halogen (2-bromo-3-methylbutane).

6. With a contact time of 0.2 second, the isotopic distribution becomes 56% at C-1, 8% at C-2, 8% at C-3, and 27% at C-4.

C. Other Preparations

2-Bromo-2-methylbutane-$C^{14}_{1/4}$ has been prepared[6] in 45% yield (b.p. 106 °) by liquid-phase isomerization[7] of 2-methylbutane-1-C^{14} over water-promoted aluminum bromide (15 hours at room temperature), followed by photochemical bromination. Degradation showed the isotopic distribution was 52% at C-1, 9% at C-2, 13% at C-3, and 26% at C-4.

[1]J. D. Roberts and R. H. Mazur, J. Am. Chem. Soc., 73, 2509 (1951).
[2]P. D. Bartlett, F. E. Condon and A. Schneider, *ibid.*, 66, 1531 (1944).
[3]C. G. Swain and L. Kent, *ibid.*, 72, 518 (1950).
[4]J. D. Roberts, R. E. McMahon and J. S. Hine, *ibid*, 72, 4237 (1950).
[5]H. M., Woodburn and F. C. Whitmore, *ibid.*, 56, 1394 (1934).
[6]J. D. Roberts and G. R. Coraor, *ibid.*, 74, 3586 (1952).
[7]H. Pines and R. C. Wackher, *ibid.*, 68, 595, 599, 1642 (1946).

1-BROMOHEXANE-1-C^{14}

$$n\text{-}C_5H_{11}C^*OOH \xrightarrow{LiAlH_4} n\text{-}C_5H_{11}C^*H_2OH \xrightarrow[H_2SO_4]{HBr} n\text{-}C_5H_{11}C^*H_2Br$$

(a) (b)

E. O. Weinman, I. L. Chaikoff, W. G. Dauben, M. Gee and C. Entenman, J. Biol. Chem., *184*, 735 (1950).

A. Procedure

(a) *1-Hexanol-1-C^{14}* (Note 1). A solution of 8.31 g. (71.6 mmoles) of hexanoic-1-C^{14} acid in 100 ml. of absolute ether is added dropwise to a stirred suspension of 143.2 mmoles of lithium aluminum hydride in 120 ml. of ether. After 15 minutes of additional stirring, the mixture is decomposed with ice water and 10% sulfuric acid. The aqueous phase is saturated with sodium chloride, separated and extracted with ether. The ether extract is washed with 4% sodium hydroxide solution, dried over sodium sulfate and concentrated; then the product is distilled; yield 6.53 g. (89.5%), b.p. 155–156°, $n_D^{24.3}$ 1.4161.

(b) *1-Bromohexane-1-C^{14}, (Hexyl-1-C^{14} Bromide)* (Note 2). A mixture of 28 g. of 48% hydrobromic acid, 7.3 g. of concentrated sulfuric acid and 6.53 g. (64 mmoles) of 1-hexanol-1-C^{14} is refluxed for 6 hours at 110–120°. The cooled mixture is diluted with water, and the bromide layer is separated, washed with concentrated sulfuric acid and water, then dried over potassium carbonate. The aqueous washes are extracted with ether, and the ether extracts are dried and combined with the bromide fraction. The solvent is removed and the product is collected by distillation; yield 8.60 g. (81.3%), b.p. 154–156°, n_D^{18} 1.4492.

B. Notes

1. The procedure is adapted from the method of Nystrom and Brown.[4] A survey of reductions with lithium aluminum hydride is presented by Brown.[5]

2. The procedure is adapted from the method of Kamm and Marvel[6] for preparing octyl bromide.

C. Other Preparations

1-Heptanol-1-C^{14} and 1-bromoheptane-1-C^{14} (heptyl-1-C^{14} bromide) have been prepared[1] in the same manner, from heptanoic-1-C^{14} acid.

The preparation of dodecyl-1-C^{14} alcohol by reduction of 2.4 g. of methyl dodecanoate-1-C^{14} at room temperature with 0.28 g. of lithium aluminum hydride in 28 ml. of absolute ether has been reported by Jerchel;[2] yield 75%, b.p. 143–144° (15 mm.).

Tetradecyl-1-C^{14} alcohol and 1-bromotetradecane-1-C^{14} have been prepared[3] similarly from the methyl ester.

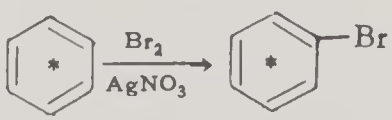
The preparation of 1-hexanol-1-C^{14} and hexyl-1-C^{14} bromide, by the method described, has been reported by Geyer.[1]

The preparation of 1-hexanol-1-C^{13} by reduction of ethyl hexanoate-1-C^{13} with lithium aluminum hydride has been reported by Crandall.[7] Hexyl-1-C^{13} bromide has been prepared by the described method.

[1]R. P. Geyer, M. Cunningham and J. Pendergast, J. Biol. Chem., *185*, 461 (1950).

[2]D. Jerchel, H. Becker and K. Schmeiser, Z. Naturforsch., *9b*, 169 (1954).
[3]G. W. Brown, Jr., D. D. Chapman, H. R. Matheson, I. L. Chaikoff and W. G. Dauben, J. Biol. Chem., *209*, 537 (1954).
[4]R. F. Nystrom and W. G. Brown, J. Am. Chem. Soc., 69, 2548 (1947).
[5]*Organic Reactions*, Vol. VI, Wiley, New York, 1951, p. 469.
[6]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 25.
[7]D. I. Crandall and S. Gurin, J. Biol. Chem., *181*, 829 (1949).

BROMOBENZENE-$C^{14}_{1/4}$



C. J. Collins and W. A. Bonner, J. Am. Chem. Soc., 77, 92 (1955).

A. Procedure (Note 1)

To a stirred, ice-cooled mixture of 65 ml. of concentrated nitric acid, 200 ml. of water and 22 ml. (222 mmoles) of benzene-C^{14}_1 is added 13 ml. (253 mmoles) of bromine, followed, dropwise in the dark (Note 2), by a solution of 44 g. of silver nitrate in 200 ml. of water during 5 hours. The mixture is treated with an excess of solid sodium sulfite (130 g.), stirred for 30 minutes and continuously extracted with ether for 16 hours, while stirring is maintained. The extract is concentrated and steam-distilled until crystals of dibromobenzene appear in the condenser; yield 34 g. The combined distillate from three identical preparations is dried over magnesium sulfate and vacuum-distilled; yield 84.3 g. (63%).

B. Notes

1. The procedure is a modification of that of Derbyshire.[1] Highly reactive solutions containing bromine cations are obtained by gradually adding silver or mercuric salts to solutions of bromine in water or acetic acid in the presence of nitric or sulfuric acid:

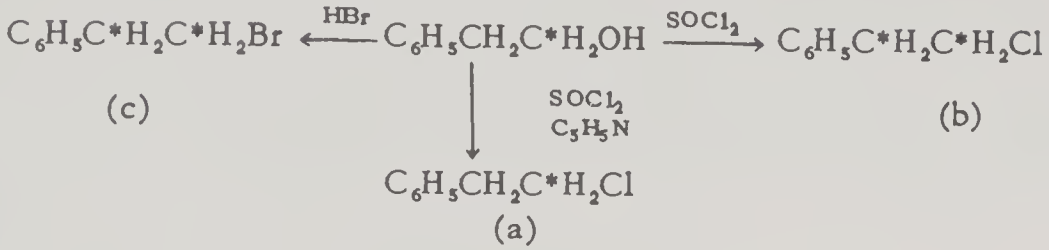
$$H_2O + Br_2 \rightleftharpoons HOBr + H^+ + Br^-$$
$$HOBr + H^+ \rightleftharpoons H_2OBr^+$$

Alkyl side chains do not appear to be attacked.

2. The apparatus may be covered with a heavy coat of black paint.

[1]D. H. Derbyshire and W. A. Waters, J. Chem. Soc., *1950*, 573.

C^{14}-(HALOETHYL)BENZENES



C. C. Lee and J. W. T. Spinks, Can. J. Chem., *32*, 1005 (1954).

A. Procedure

(a) *(2-Chloroethyl-2-C^{14})benzene, (Phenethyl-α-C^{14} Chloride)* (Note 1). To a solution of 5.00 g. of phenethyl-α-C^{14} alcohol in 20 ml. of pyridine at 0° is added slowly 10 ml. of thionyl chloride. The mixture is heated on a steam-bath for 5 minutes (Note 2), then stored at room temperature and finally poured into ice water. The product is extracted into ether solution, which is washed with water, dilute hydrochloric acid, sodium bicarbonate solution and water, then dried over sodium sulfate and distilled; yield 3.72–3.86 g. (65–67%), b.p. 85–87° (17 mm.). Degradation studies (Note 3) show that there is no isotopic rearrangement.

(b) *(2-Chloroethyl-$C^{14}_{1/2}$)benzene, (Phenethyl-α,β-$C^{14}_{1/2}$ Chloride).* To 5.00 g. of phenethyl-α-C^{14} alcohol is added slowly 15 ml. of thionyl chloride with ice-bath cooling. The mixture is refluxed for 30 minutes and fractionated under reduced pressure; yield 4.40–4.64 g. (77–81%), b.p. 81–84° (14 mm.). Degradation studies (Note 4) indicate that 48.6–50.3% of the initial activity is found at C-2 as a result of rearrangement.

(c) *(2-Bromoethyl-$C^{14}_{1/2}$)benzene, (Phenethyl-α,β-$C^{14}_{1/2}$ Bromide).* A mixture of 5.00 g. of phenethyl-α-C^{14} alcohol and 28 g. of 48% hydrobromic acid is refluxed for 2 hours. The cooled mixture is poured into ice water and extracted with ether. The extract is washed with water and sodium bicarbonate solution, then dried over sodium sulfate and concentrated, and the product is distilled; yield 6.05–6.30 g. (80–83%), b.p. 110–113° (17 mm.). Degradation studies indicate that about 6% of the initial activity is found at C-2 as a result of isotopic rearrangement.

B. Notes

1. The replacement of a hydroxyl group with chlorine, without rearrangement, by the use of thionyl chloride in pyridine was reported by Whitmore.[1] The reaction is probably less time-consuming, and may give higher yields, than the sulfonate-sodium halide method for preparing unrearranged alkyl halides; see allyl-1-C^{14} bromide.

2. Prolonged heating results in extensive decomposition.

3. Directions are given by Lee for preparing hydrocinnamic-α-C^{14} acid, (3-phenylpropionic-2-C^{14} acid) m.p. 48–49°, in 70–80% yield by carbonation of the Grignard reagent with Dry Ice. Oxidation with alkaline permanganate solution gives nonisotopic benzoic acid (80%).

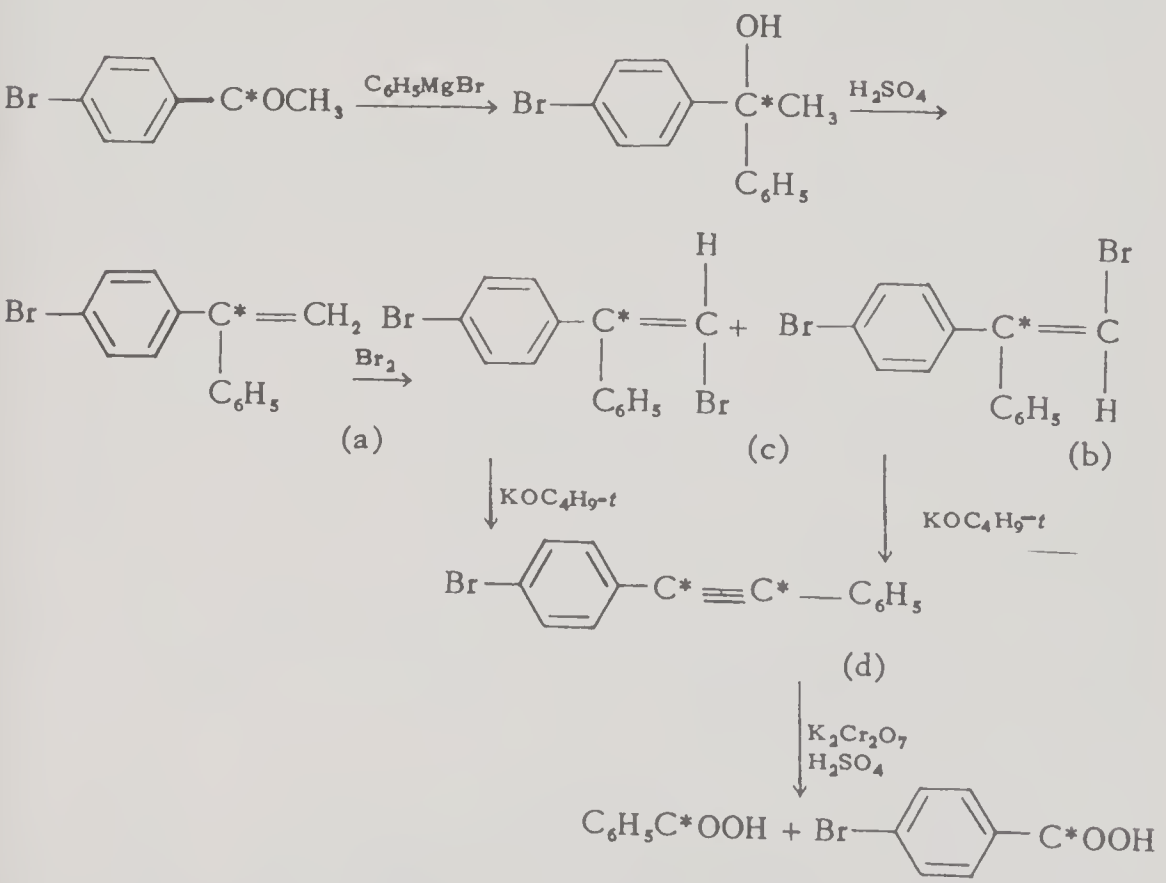
4. Carbonation of the Grignard reagent forms hydrocinnamic-α,β-$C^{14}_{1/2}$ acid and oxidation of the acid, as above, forms benzoic-C^{14} acid containing approximately one-half of the initial molar specific activity.

C. Other Preparations

In the same manner is prepared (2-iodoethyl-2-C^{14})benzene from 38 g. of 55% hydriodic acid; yield 90–92%, b.p. 115–118° (14 mm.). Degradation studies indicate that 2.7% of the initial activity is found at C-2. Halogenation with red phosphorus and iodine gave the same product (81–85%) with about 6% of the initial activity located at C-2.

[1]F. C. Whitmore, F. A. Karnatz and A. H. Popkin, J. Am. Chem. Soc., 60, 2540 (1938).

p-BROMOPHENYLPHENYLACETYLENE-$C^{14}_{1/2}$
(4-Bromotolan-α,β-$C^{14}_{1/2}$)



A. A. Bothner-By, J. Am. Chem. Soc., 77, 3293 (1955).

A. Procedure

(a) *1-(p-Bromophenyl)-1-phenylethylene-1-C*^{14} (Note 1). To the Grignard solution, prepared from 5.5 g. of bromobenzene, 1.0 g. of magnesium and 40 ml. of ether, is added dropwise a solution of 3.49 g. of 4′-bromoacetophenone-1-C^{14} in 20 ml. of ether. The mixture is refluxed overnight, hydrolyzed by the slow addition of 10% sulfuric acid and refluxed for 4 hours. The aqueous layer is extracted with 20 ml. of ether, and the combined extract is dried over sodium sulfate and evaporated on a steam-bath; yield 4.65 g. (nearly quantitative).

(b) cis-*2-Bromo-1-(p-bromophenyl)-1-phenylethylene-1-C*^{14} (Note 2). To a stirred solution of the 1-(*p*-bromophenyl)-1-phenylethylene-1-C^{14} in 20 ml. of carbon disulfide is added dropwise a solution of 0.93 ml. of bromine in 10 ml. of carbon disulfide (Note 3). The solvent is removed in a steam-bath, and the residue is crystallized overnight from 10 ml. of ligroin (b.p. 60–90°). The large crystals of high-melting isomer (107°) are collected, and 0.5 g. of carrier is recrystallized from the mother liquor (1 week). The combined crops are recrystallized twice from ligroin; yield 1.10 g. (18%), m.p. 106.0–106.4° (Note 4).

(c) trans-*2-Bromo-1-(p-bromophenyl)-1-phenylethylene-1-C*^{14}. The above mother liquor is diluted with 50 ml. of petroleum ether and passed through a 1 × 10-cm. column of alumina (Note 5). Elution with ten 50 ml. portions of distilled petroleum ether and slow evaporation of the fractions result in the formation of solids in the first three. The combined oily material from all fractions is diluted with 10 ml. of ligroin (b.p. 60–90°), and from this solution are recrystallized six successive 0.4-g. portions of carrier 107° isomer (Note 6). The mother liquor is evaporated, and the residual oil is diluted with 4 ml. of petroleum ether, seeded and stored for 2 weeks; yield 0.26 g. (4%), m.p. 42–44° (Note 7).

(d) p-*Bromophenylphenylacetylene-C*$^{14}_{1/2}$. To a solution of 0.150 g. of potassium (1.5 equivalents) in 5 ml. of *t*-butyl alcohol is added 0.95 g. of 2-bromo-1-(*p*-bromophenyl)-1-phenylethylene-1-C^{14}. The solution is refluxed for 3 days, then diluted with 20 ml. of water and 10 ml. of *t*-butyl alcohol, warmed to solution and cooled, yielding 0.71 g. (nearly quantitative) of slightly yellowish flat plates. The product is washed with cold methanol and recrystallized from ligroin (b.p. 60–90°); m.p. 83–85°. Degradation studies (Note 9) indicate that 88% and 8% of the activity is located at C-1 in the products derived from the *cis*- and *trans*-starting materials, respectively.

B. Notes

1. The method is similar to that of Allen and Converse.[1]

2. The method is that of Stoermer and Simon.[2] The assignment of geometrical configuration to the 107° and 43° melting isomers is that

of Bergmann,[3] rather than Stoermer,[2] the argument being based on dipole moment measurements. The infrared spectra are given.

3. The bromine reacts as fast as added, and hydrogen bromide is evolved.

4. The high-melting isomer crystallizes in the form of small truncated pyramids with sharply defined facets.

5. Merck Aluminum Oxide, "suitable for chromatographic absorption". Colored impurities are left on the column.

6. The last crop shows no activity after two recrystallizations.

7. The low-melting isomer crystallizes in the form of flat needles. A few crystals of the contaminating high-melting isomer are removed by hand-picking, but do not interfere with any tracer work.

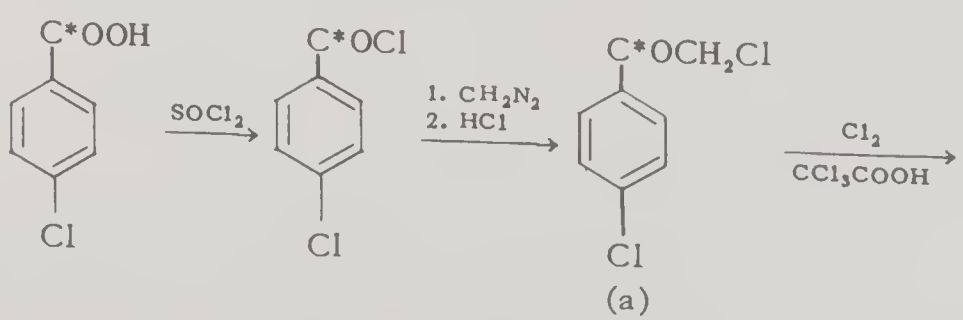
8. The preparation is incidental to a determination of the stereospecificity of, and a study of the mechanism of the Fritsch-Buttenberg-Wiechell rearrangement[4] of haloethenes, which is initiated by strong base: $(AR)_2C{=}CHX \rightarrow (AR)C{\equiv}C(AR)$.

9. Directions are given by Bothner-By for oxidizing *p*-bromophenylphenylacetylene-$C^{14}_{1/2}$ with chromic acid, forming benzoic-C^{14} acid (m.p. 120–121°) and *p*-bromobenzoic-C^{14} acid (m.p. 251–252°), which are separated by sublimation at different pressures, and recrystallization from water. The *cis*- and *trans*-ethylenic compounds rearrange with 88% migration of the phenyl group and 92% migration of the *p*-bromophenyl group, respectively.

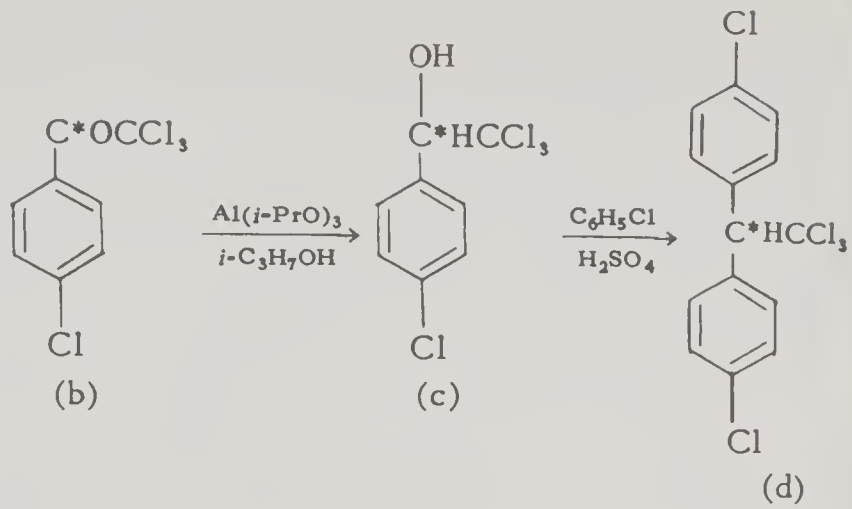
[1]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 226.
[2]R. Stoermer and M. Simon, Ann., *342*, 1 (1905).
[3]E. Bergmann, L. Engel and H. Meyer, Ber., *65B*, 446 (1932).
[4]G. H. Coleman and R. D. Maxwell, J. Am. Chem. Soc., *56*, 132 (1934); G. H. Coleman, W. H. Holst and R. D. Maxwell, *ibid*, 58, 2310 (1936).

1,1,1-TRICHLORO-2,2-BIS(*p*-CHLOROPHENYL)ETHANE-2-C^{14} (C^{14}_1-DDT)

METHOD I



(a)



D. R. Buhler, C. H. Wang and B. E. Christensen, J. Am. Chem. Soc., 75, 4336 (1953).

A. Procedure (Note 1)

(a) *2, 4′-Dichloroacetophenone-1-C*^{14}, (*Chloromethyl* p-*Chlorophenyl Ketone-C*^{14}). To a stirred, ice-cooled solution of 20 ml. of anhydrous benzene and *p*-chlorobenzoyl-C^{14} chloride, prepared from 0.9501 g. of *p*-chlorobenzoic-C^{14} acid and thionyl chloride, is added slowly an ethereal solution of 34 mmoles of diazomethane ir small portions. The mixture is stored overnight at room temperature, then cooled in an ice-bath and treated with dry hydrogen chloride for 1 hour. The solvents are evaporated under reduced pressure, and the crude product is crystallized from aqueous dioxane; yield 0.5089 g. (44.5%), m.p. 99.0–99.5°.

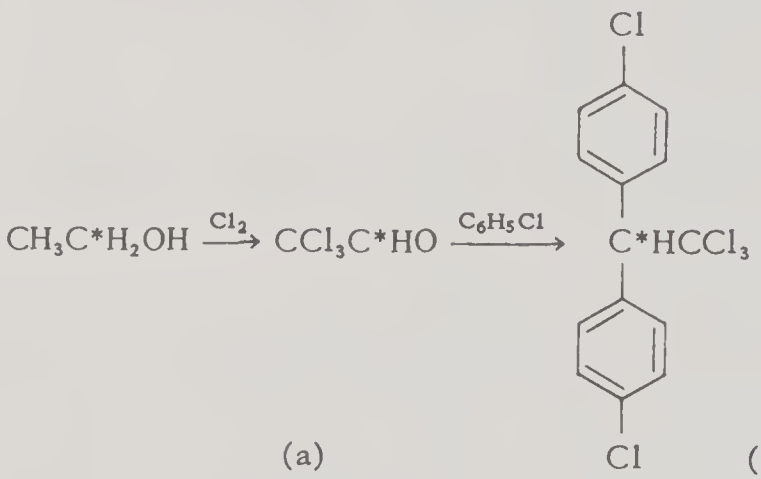
(b) *2,2,2,4′-Tetrachloroacetophenone-1-C*^{14}, (*Trichloromethyl* p-*Chlorophenyl Ketone-C*^{14}). The chloromethyl *p*-chlorophenyl ketone-C^{14}, dissolved in an equal weight of trichloroacetic acid, is chlorinated at 120–125° for 48 hours with a stream of chlorine (Note 2). The warm mixture is poured into 25 ml. of cold water and neutralized with 4% sodium carbonate solution. The product is extracted into benzene (Note 3), and the solvent is evaporated.

(c) *1-(*p-*Chlorophenyl)-2, 2, 2-trichloroethanol-1-C*^{14}. To a solution of the crude ketone in 100 ml. of dry isopropyl alcohol is added 14.8 mmoles of freshly prepared aluminum isopropoxide,[1] with slow distillation of the acetone formed. After 8 hours (Note 4), the mixture is evaporated under reduced pressure, and the residue is hydrolyzed with 2 *N* hydrochloric acid. The product is extracted into benzene in a continuous extractor (12 hours), and the solvent is evaporated; yield 70%, based on 2,4′-dichloroacetophenone (Note 5).

(d) *1, 1, 1-Trichloro-2, 2-bis*(p-*chlorophenyl)ethane-2-C*^{14}. To the crude carbinol is added 4 ml. (39 mmoles) of anhydrous chlorobenzene and 4 ml. (76 mmoles) of 98.5% sulfuric acid. The mixture is stirred at room temperature for 40 hours, with addition of 4 ml. of "101%" sulfuric

acid during the first 2 hours. The mixture is finally poured into water and extracted continuously for 10 hours with petroleum ether. The solvent is evaporated, and the residue is dissolved in 100 ml. of propyl alcohol. The solution is concentrated to 20 ml. (Note 6), diluted with an equal volume of water and cooled to crystallize the crude product (0.7 g.). Purification is achieved by recrystallization from ethanol and petroleum ether—ethanol; yield 0.2412 g., m.p. 104–104.5° (Note 7).

METHOD II



G. W. Pearce and J. A. Jensen, Science, *118*, 45 (1953); J. Agr. Food Chem., *1*, 776 (1953).

A. Procedure

(a) *Chloral-1-C*^{14} (Note 8). Chlorine is passed through 4.10 g. (89 mmoles) of ethanol-1-C^{14} at the rate of 10 ml. per minute. The exothermic reaction raises the temperature of the reaction mixture to a maximum of 41° during the first hour. A cold finger chilled with refrigerated water is used as a reflux condenser, and unreacted chlorine is collected in a trap at −79°. After 5 hours, the temperature of the reaction mixture is raised to 50–60° for 3 hours, then to 90° for 24 hours to complete the chlorination (5–10 ml. per minute). The mixture is cooled to room temperature and freed of chlorine by sweeping of the system with nitrogen. The product is distilled under vacuum into a dry trap cooled with liquid nitrogen (Note 9); then the vacuum is displaced by dry nitrogen; yield 7.93 g., 4.9 ml. (Note 10).

(b) *1,1,1-Trichloro-2,2-bis*(p-*chlorophenyl*)*ethane-2-C*^{14} (Note 11). To a stirred, ice-cooled mixture of the crude chloral-1-C^{14} and 20 ml. (197 mmoles) of chlorobenzene is added 60 ml. of 99% sulfuric acid, followed by 60 ml. of 101% sulfuric acid in 2 ml. portions at 10-minute intervals while the mixture is maintained at 0–5°. When a precipitate forms (1.5 hours), the mixture is stored in the cold for 10 hours. The product is collected and extracted into hexane solution, which is washed with 4% sodium carbonate solution and water, then evaporated and dried

under vacuum at 90°; yield 15.83 g., 42% based on ethanol. Two recrystallizations from ethanol yield 6.11 g. of product, m.p. 107–107.5° (Note 12).

B. Notes

1. The procedure is an adaptation of the method proposed by Fry.[2] Gunther and Blinn[3] have outlined a number of reactions of potential use in preparing labeled DDT analogues and intermediates.

2. The modified Pregl methoxyl apparatus described by Christensen[4] is employed as the reactor.

3. A continuous extractor is used for 16 hours.

4. No acetone is detected after 2 hours (2,4-dinitrophenylhydrazine).

5. The acetate melts at 123–124°. A similar isotopic preparation is described by Macdonald,[5] the ketone being prepared by chlorination of 4′-chloroacetophenone-1-C^{14} at 210°.

6. Any unreacted chlorobenzene is removed as the azeotrope.

7. The over-all yield and radiochemical yield are 10% based on barium carbonate. The evaporated mother liquor yields an oil which is presumed to be a mixture of isomers of DDT. A similar isotopic preparation is described by Macdonald.[5] The over-all radiochemical yield, by a slightly different route up to (b), was 4.8% based on barium carbonate.

8. The chlorination is carried out in a specially designed flask; see Figure IX, 3. The method is outlined by Thorpe.[6] No heat is applied for the first 5 hours.

9. Steam is passed through the cold finger, and the flask is heated at 90–95°; then the flask and lines are swept with a luminous flame for 10 minutes to complete the vacuum transfer.

10. The crude product contains some chloral alcoholate and a small amount of chloral hydrate, depending upon the dryness of reagents and apparatus. Incomplete chlorination gives dichloroacetaldehyde, b.p. 88° (oxime, m.p. 177–178°).

11. The procedure is an adaptation of the method studied in detail by Mosher,[7] and which gives DDT in yields of 95–98% (75–80% purity, m.p. 80–94°).

12. The radiochemical yield is 17% based on barium carbonate. The mother liquor may be treated for recovery of additional product as well as the *o*, *p*′-isomer, 1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-ethane[-2-C^{14}], m.p. 73–74° (approximately 15%)[6].

[1] *Organic Reactions*, Vol. II, Wiley, New York, 1944, p. 178.

[2] E. M. Fry, J. Am. Chem. Soc., *71*, 3238 (1949).

[3] F. A. Gunther and R. C. Blinn, J. Chem. Ed., *27*, 654 (1950).

[4] B. E. Christensen, L. Friedman and Y. Sato, Ind. Eng. Chem., Anal. Ed., *13*, 276 (1941).

[5] R. Macdonald and B. E. Baker, Can. J. Chem., *31*, 517 (1953).

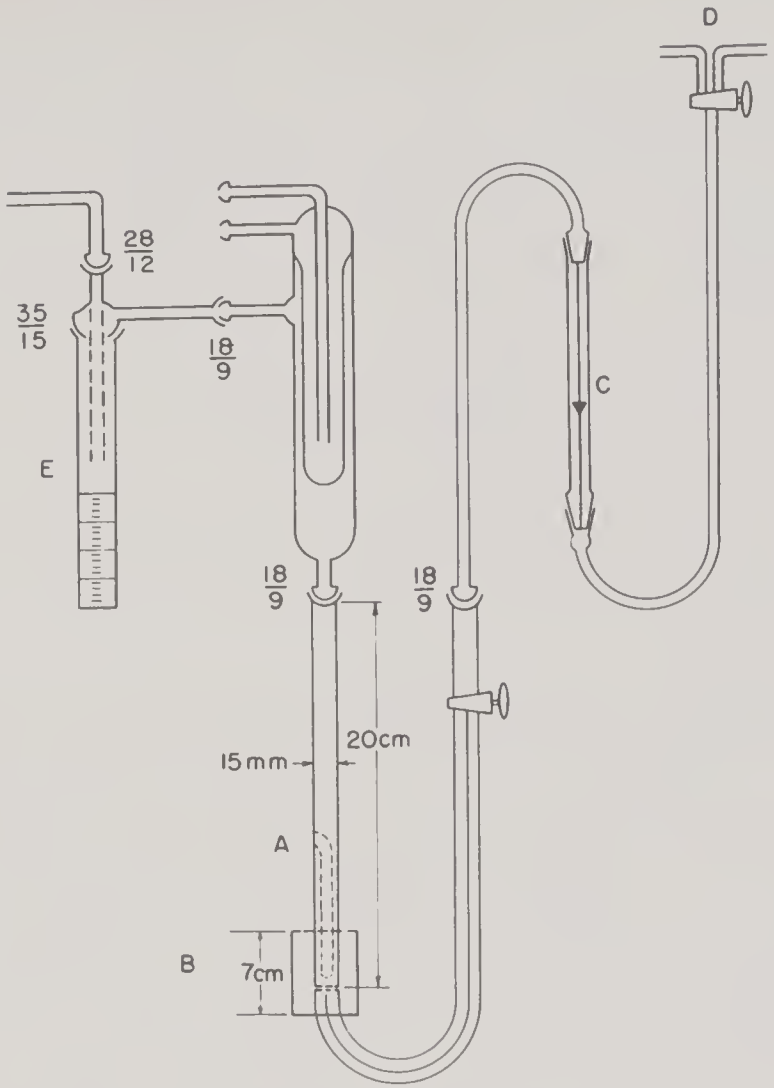
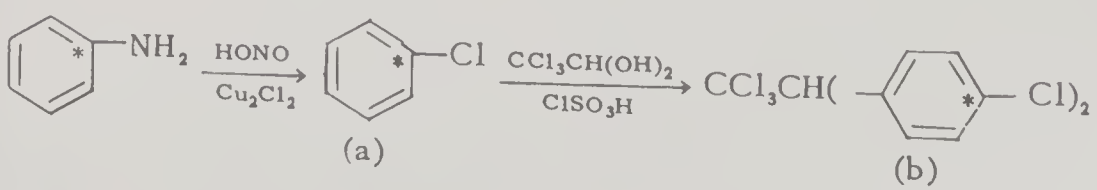


Fig. IX, 3. Chlorination apparatus (G. W. Pearce and J. A. Jensen). A, reaction chamber with a thermocouple well and a sintered glass disc (porosity C); B, 250-W heating coil; C, flowrator; D, inlets for chlorine and nitrogen; E, trap (8 ml.); all stopcocks are 2 mm.

[6]E. Thorpe, *Dictionary of Applied Chemistry*, Vol. 2, Longmans, New York, 1921, p. 180.

[7]H. S. Mosher, M. R. Cannon, E. A. Conroy, R. E. Van Strien and D. P. Spalding, Ind. Eng. Chem., *38*, 916 (1946).

1,1,1-TRICHLORO-2,2-BIS(4-CHLOROPHENYL-4-C^{14})ETHANE (C_2^{14}-DDT)



(a) (b)

M. Fields, J. Gibbs and D. E. Walz, Science, *112*, 591 (1950).

A. Procedure

(a) *1-Chlorobenzene-1-C^{14}.* The reaction vessel is a 500-ml. three-necked flask equipped with a mercury-sealed stirrer, a pressure-equalizing dropping funnel and a reflux condenser connected to a trap cooled with liquid nitrogen. To a hot solution of cuprous chloride, freshly prepared from 15.6 g. of copper sulfate, in 22 ml. of concentrated hydrochloric acid is added at a moderate rate a cold solution of diazonium salt, prepared from 6.50 g. (50 mmoles) of aniline-1-C^{14} hydrochloride and nitrous acid at −10°. The mixture is stirred for 15 minutes without external heating and then is extracted with ether, the condenser and trap being rinsed to recover droplets carried over by the rapid evolution of nitrogen. The ether solution is washed with alkali and water, dried over calcium chloride, concentrated and distilled; yield 4.12 g. (73%), b.p. 125–129.5° (Note 1).

(b) *1,1,1-Trichloro-2,2-bis(4-chlorophenyl-4-C^{14})ethane.* The reaction vessel is a 100-ml. three-necked flask equipped with a mercury-sealed stirrer, a 5-ml. pressure-equalizing dropping funnel and a drying tube filled with potassium hydroxide. To a rapidly stirred solution of 2.98 g. (19 mmoles) of chloral hydrate and 4.05 g. (36 mmoles) of 1-chlorobenzene-1-C^{14}, cooled in an ice-bath, is added slowly 2.32 ml. (36 mmoles) of freshly distilled chlorosulfonic acid at the rate of 1–2 drops per second over a period of about 90 minutes. The ice-bath is removed, and the mixture is stirred for 20 hours, then diluted with a few ml. of ice water and extracted with a total of 30–40 ml. of carbon tetrachloride. The organic solution is washed with cold water, 2% sodium carbonate solution and cold water, then dried and concentrated under vacuum. The residue is crystallized from 15 ml. of 95% ethanol, giving a 75–85% yield of crude product, m.p. 97–102°. Recrystallization gives the pure product (40–50% yield), m.p. 108.5–109.5° (Note 2).

B. Notes

1. $n_D^{26.5}$ 1.5198. The yield is usually 68–75%. Chlorobenzene-$C^{14}_{1/4}$ may be prepared (42–65% yield) by reacting equimolar amounts of benzene-C^{14}_1 and chlorine in a sealed tube at 0–30° in the presence of an iron-iodine catalyst. This product would not be labeled at any definite position, and the method does form some poly-halogenated compounds. The yield becomes 80–90% in the presence of excess benzene.

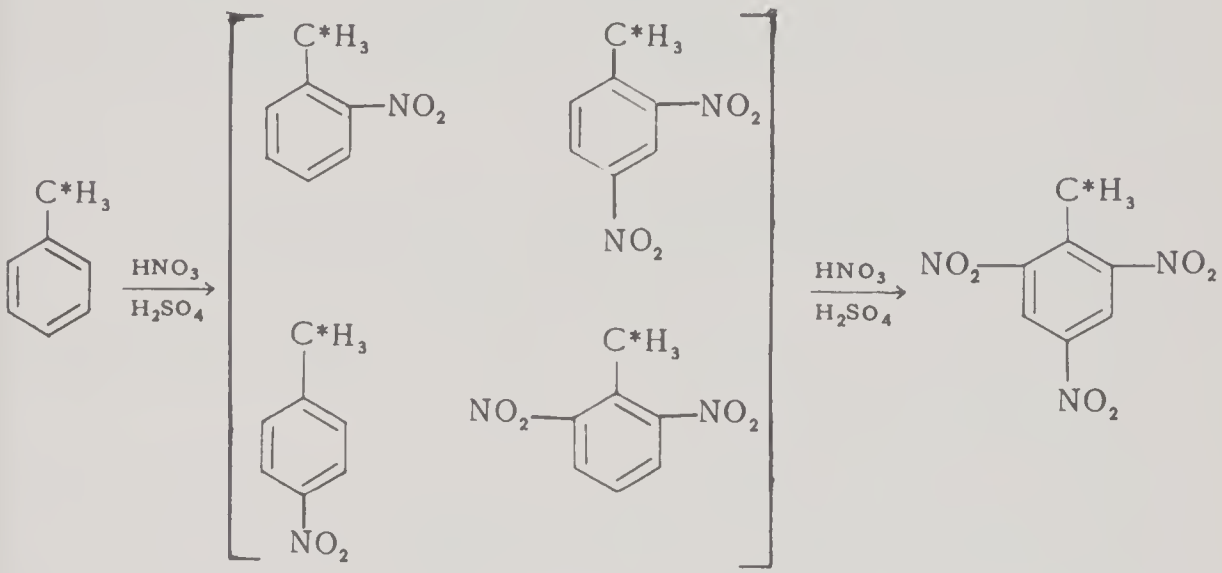
2. The procedure is adapted from the method of Cook[1], who obtained yields of 62% (m.p. 104–105°) on a large scale. The yield is not improved by employing chloral itself, nor by using a 10% excess of chlorobenzene. The yield is 45%, m.p. 106.5–107.5°, when chloral is reacted

with a 10% excess of chlorobenzene in the presence of sulfuric acid by the method of Mosher.[2]

[1]W. A. Cook, K. H. Cook and W. H. C. Rueggeberg, Ind. and Eng. Chem., 39, 868 (1947).

[2]H. S. Mosher, M. R. Cannon, E. A. Conroy, R. E. Van Strien and D. P. Spalding, *ibid.*, 38, 916 (1946).

2,4,6-TRINITROTOLUENE-α-C^{14} (α-TNT-α-C^{14})



A. Murray III, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, unpublished work.

A. Procedure (Note 1)

The apparatus consists of a 10-ml. round-bottomed flask equipped with a glass enclosed magnetic stirring bar, a sealed-on reflux condenser, and attachment to a vacuum manifold. To 0.323 g. (3.5 mmoles) of crude toluene-α-C^{14} is added dropwise, with stirring and ice-bath cooling, 1.20 ml. of cold mixed acid. The acid is prepared by adding slowly 16.3 ml. of concentrated sulfuric acid to 14.1 ml. of concentrated nitric acid with stirring and cooling. The reaction mixture is stirred overnight at room temperature; then the temperature is raised to 50° during 1.5 hours and maintained there for 1 hour. To the stirred mixture is added slowly 0.65 ml. of yellow fuming nitric acid (d. 1.50), followed by 1.70 ml. of fuming sulfuric acid (d. 1.92, 20% SO_3) with ice-bath cooling (Note 2). The mixture is heated gradually to 120° over a period of 9 hours, then cooled

and poured onto 16 g. of crushed ice (Note 3). The nearly colorless crude product is collected, washed with a few ml. of cold water and dried in a vacuum desiccator; yield 32.3% (Note 4). Purification is accomplished by dissolution in alcohol, treatment with carbon and crystallization at 0° (Note 5). The recrystallized compound melts at 80.5–81.5° (Note 6).

B. Notes

1. The procedure is a modification of the method described by Hickinbottom[1] and by Gibson[2] for preparing the mono-, di- and tri-nitrotoluenes; also see *o*- and *p*-nitrotoluene-α-C^{14}. The method is adapted to an isotopic synthesis by a two-stage nitration without isolation of intermediates.

2. On the assumption that reaction has taken place to the extent of 60% mono- and 40% disubstitution, the fortified acid mixture consists of 17.5% HNO_3, 78.0% H_2SO_4 and 4.5% H_2O.

3. The product is recovered most efficiently from 4–5 volumes of water.[3]

4. In trial runs starting with pure toluene, the yield of crude product (m.p. 77–79°) was nearly quantitative.

5. The solubility of TNT per g. of 95% alcohol is 0.195 g. at 75° and 0.0065 g. at 0°. The chief impurities in crude TNT prepared under the usual conditions of nitration[2,5] are: 3,4,6-TNT (2.9%), 2,3,4-TNT (1.3%), 2,3,6-TNT (0.3%), and unconverted dinitrotoluenes. Purification by crystallization from alcohol[5] is superior to the sulfite method,[5] which relies upon dissolution of asymmetrical tolites in cold sodium sulfite solution by replacing a labile *m*-nitro group.

6. In trial runs with pure toluene, the yield of purified product was 72–76%. TNT decomposes above 150°.

C. Other Preparations

By the same procedure, starting with toluene-1-C^{14}, is prepared 2,4,6-trinitrotoluene-1-C^{14}; yield 76.6% (m.p. 80.5–81.5°) at the 45-mmole scale.

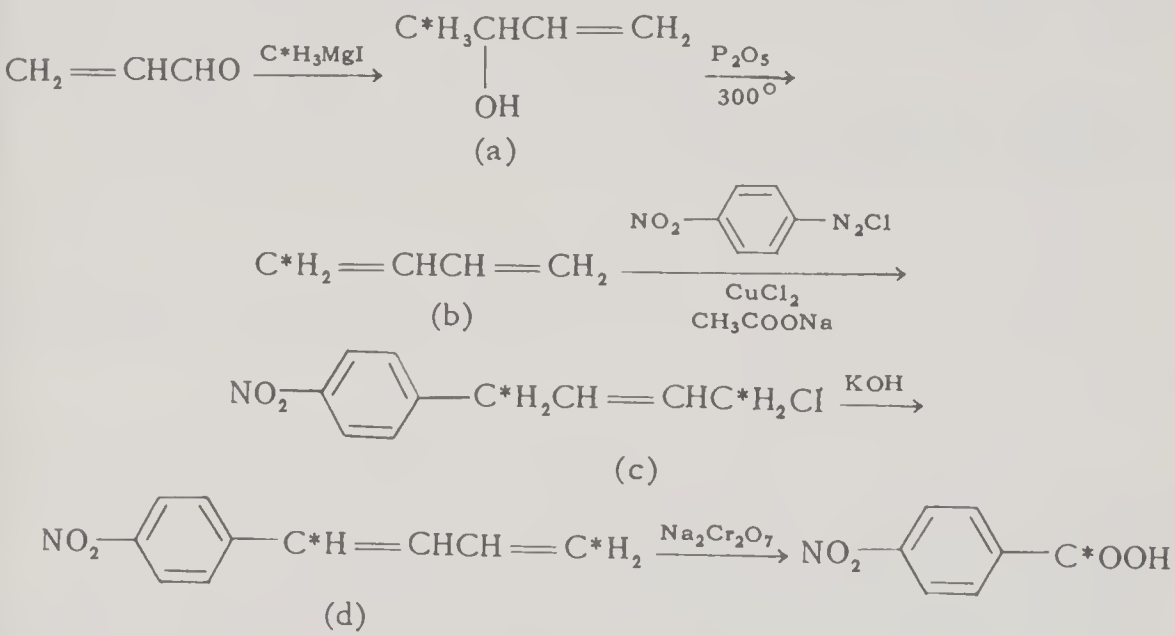
The preparation of *o*-nitrotoluene-α-C^{14} and *p*-nitrotoluene-α-C^{14} by nitration of toluene-α-C^{14} in acetic anhydride has been reported by Wheland[6]. The isomers were separated by distillation.

The preparation of nitrobenzene-$C^{14}_{1/4}$ has been described by Parke.[7] To a mixture of 10 g. of nitric acid and 15 g. of sulfuric acid is added slowly 5.0 g. of benzene-C^{14}_1, while the temperature is maintained below 45°. The mixture is stored at 0° for 30 minutes and the aqueous layer is separated, diluted to 100 ml. with water and extracted with two 10-ml.

portions of ether. The combined extract and crude product is washed with 10 ml. of water and two 10 ml. portions of 10% ammonium chloride solution, then dried over calcium chloride and concentrated, and the product is distilled; yield 6.4 g. (69%), b.p. 208–211°. The nonvolatile residue was shown by isotopic dilution technique to contain 24.5% of the starting material as 1,3-dinitrobenzene-1,2,4,5-$C^{14}_{1/4}$, 2.4% as 1,2-dinitrobenzene-1,3,4-$C^{14}_{1/3}$ and 0.25% as 1,4-dinitrobenzene-1,2-$C^{14}_{1/2}$.

[1]W. J. Hickinbottom, *Reactions of Organic Compounds*, Longmans, Green and Co., London, 1936, p. 37.
[2]W. H. Gibson, R. Duckham and R. Fairbairn, J. Chem. Soc., *121*, 270 (1922).
[3]W. McHutchison and R. Wright, J. Soc. Chem. Ind., *34*, 781 (1915).
[4]M. Coprisarow, Chem. News, *113*, 37 (1916).
[5]H. Muraour, Bull. soc. chim. France, (4) *35*, 367 (1924).
[6]G. W. Wheland and J. R. Schwartz, J. Chem. Phys., *17*, 425 (1949).
[7]D. V. Parke, Biochem. J., *62*, 339 (1956).

1-(*p*-NITROPHENYL)-1,3-BUTADIENE-1,4-$C^{14}_{1/2}$



G. A. Ropp and V. F. Raaen, J. Am. Chem. Soc., 76, 4484 (1954).

A. Procedure

(a) *3-Buten-2-ol-1-C^{14}.* The isotopic preparation is made by some suitable adaptation of the Grignard reaction. A representative procedure is that of Prevost,[1] which follows.

To a shaken mixture of 25 g. (1 mole) of magnesium turnings and 200 ml. of absolute ether, cooled with an ice-salt-bath, is added dropwise a solution of 1.2 moles of methyl bromide in 5 volumes of ether cooled to −10°. To the shaken Grignard solution is added slowly 0.9 mole of pure

acrolein in 5–6 volumes of ether at −20° during 3–4 hours. The temperature is allowed to rise, and the mixture is stored for 18 hours and then decomposed by the dropwise addition of nearly 1 l. of saturated ammonium chloride solution. The aqueous solution is separated and distilled until the vapor temperature reaches 100°. The distilled azeotrope is saturated with potassium carbonate and extracted with ether. The combined ether solution is washed with dilute hydrochloric acid and dried over potassium carbonate, then concentrated by distillation through a column; yield 60% (b.p. 96–97°) based on acrolein (Note 1).

(b) *1,3-Butadiene-1-C*^{14}. 3-Buten-2-ol-1-C^{14} is dehydrated at 300° over phosphorus pentoxide (Note 2). Butadiene may be dried by passage through calcium chloride and condensed[1a] in a trap at −78°, b.p. −4° to −5°.

(c) *4-Chloro-1-(p-nitrophenyl)-2-butene-1,4-C*$^{14}_{1/2}$ (Note 3). 1,3-Butadiene-1-C^{14} is added to *p*-nitrobenzenediazonium chloride with elimination of nitrogen, according to the procedure of Ropp and Coyner.[2] Degradation studies indicate that one half of the activity is located at C-1 (Note 4).

(d) *1-(p-Nitrophenyl)-1,3-butadiene-1,4-C*$^{14}_{1/2}$. The chlorobutene is dehydrohalogenated by boiling with alcoholic alkali according to the procedure of Ropp and Coyner.[2] The yield is essentially quantitative.

B. Notes

1. D_4^{20} 0.8318, $n_D^{26.5}$ 1.4087. 3-Buten-2-ol *p*-nitrobenzoate, crystallized from alcohol, melts at 43–44°.

2. For similar preparations see Wagner and Zook.[3]

3. The reaction is an extension[2] of the Meerwein[4] condensation, or the addition of diazonium compounds to α,γ-unsaturated carbonyl compounds with loss of nitrogen. The preparation is incidental to a study of the isotope effect with an asymmetrically-labeled symmetrical molecule.

4. *p*-Nitrobenzoic-C^{14} acid is prepared by refluxing for 3 hours a mixture of 2.0 g. of 4-chloro-1-(*p*-nitrophenyl)-2-butene-1,4-C$^{14}_{1/2}$, 50 ml. of water, 15 g. of sodium dichromate and 15 ml. of concentrated sulfuric acid. The molar specific activity is 50.8% of that of the starting material; yield nearly quantitative. *p*-Nitrobenzoic acid melts at 242°.

[1]C. Prevost, Ann. chim., (10) *10*, 147 (1928); A. Wohl and M. S. Losanitsch. Ber., *41*, 3621 (1908).

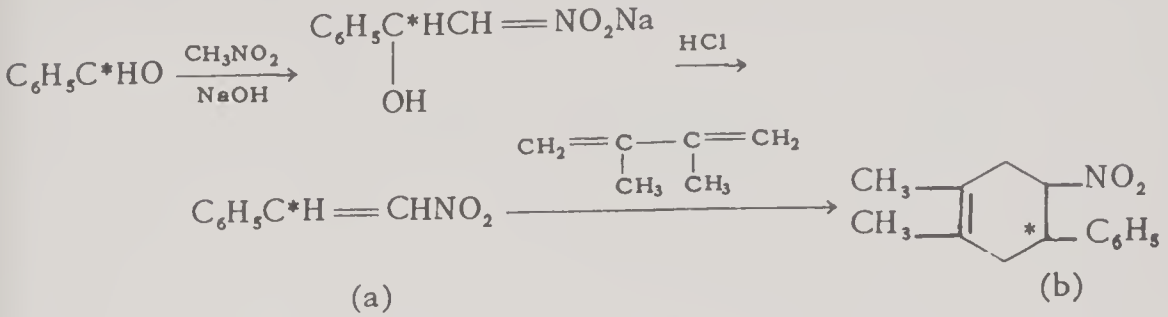
[1a]M. S. Kharasch, E. T. Margolis and F. R. Mayo, J. Org. Chem., *1*, 393 (1936).

[2]E. C. Coyner and G. A. Ropp, J. Am. Chem. Soc., *70*, 2283 (1948); *Organic Syntheses*, Vol. 31, Wiley, New York, 1951, p. 80.

[3]R. B. Wagner and H. D. Zook, *Synthetic Organic Chemistry*, Wiley, New York, 1953, p. 32.

[4]H. Meerwein, E. Büchner and K. van Emster, J. prakt. Chem., (2) *152*, 237 (1939); E. Müller, Angew. Chem., *61*, 179 (1949).

1,2-DIMETHYL-5-NITRO-4-PHENYLCYCLOHEXENE-4-C^{14}



G. A. Ropp, V. F. Raaen and A. J. Weinberger, J. Am. Chem. Soc., 75, 3694 (1953).

A. Procedure

(a) *β-Nitrostyrene-α-C^{14}.* Benzaldehyde-C^{14} is condensed with nitromethane[1] in alkaline solution by the procedure of Worrall.[2] The yield of recrystallized product on the 5-mole scale is 80–83%, m.p. 57–58°. Also see 1,3-dihydroxy-2-propanone-2-C^{14}, Note 4.

(b) *1,2-Dimethyl-5-nitro-4-phenylcyclohexene-4-C^{14}* (Note 1). A mixture of 30 mmoles of 2,3-dimethyl-1,3-butadiene,[3] 20 mmoles of β-nitrostyrene-α-C^{14} and 20 ml. of anhydrous toluene is refluxed overnight. The solvent and excess diene are evaporated under vacuum, and the residue is recrystallized from dilute ethanol. The product melts at 91.5–92.5° after vacuum sublimation (Note 2).

B. Notes

1. The method is that of Allen,[4] who obtained the adduct in 82% yield.

2. There is no isotope effect in this Diels-Alder addition. The determination was made by comparing the specific activities of the products obtained by (a) complete reaction, as illustrated here, and (b) partial reaction (30%), obtained by reacting 50 mmoles of the styrene with only 15 mmoles of the diene. Experiment has demonstrated that this particular Diels-Alder reaction is not reversible, for there is no isotopic exchange when equimolecular amounts of β-nitrostyrene-α-C^{14} and nonisotopic adduct are melted together for 24 hours at 130°.

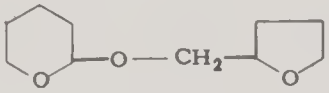
[1] *Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 401.
[2] *Ibid.*, Coll. Vol. I, 1941, p. 413.
[3] *Ibid.*, Vol. 22, 1942, p. 39.
[4] C. F. H. Allen, A. Bell and J. W. Gates, Jr., J. Org. Chem., 8, 373 (1943).

HYDROXY COMPOUNDS

METHANOL-C^{14}

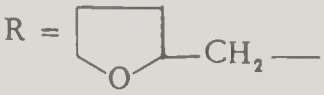
METHOD I

$$4\ C^*O_2 + 3\ LiAlH_4 \longrightarrow LiAl(OC^*H_3)_4 + 2\ LiAlO_2$$



$$(LiAlH_4 + 4\ ROH \longrightarrow LiAl(OR)_4 + 4\ H_2)$$

$$LiAl(OC^*H_3)_4 + 4\ ROH \xrightarrow[\Delta]{} LiAl(OR)_4 + 4\ C^*H_3OH$$



J. D. Cox and R. J. Warne, Nature, *165*, 563 (1950); J. D. Cox, H. S. Turner and R. J. Warne, J. Chem. Soc., *1950*, 3167.

A. Procedure (Note 1)

An 0.8 *M* solution of lithium aluminum hydride (2 molar equivalents) in tetrahydrofurfuryloxytetrahydropyran (Note 2), contained in a flask equipped with a magnetic stirrer, distilling head with dephlegmator, a receiver-trap and attachment to a high-vacuum manifold, is cooled in an ice-bath and pumped on briefly to remove dissolved gases (Note 3). The flask is cooled in Dry Ice, evacuated to about 1 μ and closed off from the manifold. The reducing mixture is stirred in an ice-bath, while dry carbon-C^{14} dioxide (3–15 mmoles) is admitted from a reservoir. When the absorption is complete (1 hour), nitrogen is admitted to the system, and there is attached to the flask a pressure-compensating dropping funnel equipped with a nitrogen inlet. With the trap cooled in liquid nitrogen, 12 molar equivalents of tetrahydrofurfuryl alcohol are added dropwise to the stirred

mixture in a slow stream of dry nitrogen. The mixture is heated at 110° for 2–4 hours, and the product, released by alcoholysis, together with some solvent is distilled into the trap under conditions of partial reflux at atmospheric pressure in a slight current of inert entraining gas (Note 4). The product is vacuum-redistilled at room temperature into a receiver cooled with liquid nitrogen; yield about 90% (Note 5).

METHOD II

$$HC^*OOH \xrightarrow[10\%\ Ni(OH)_2]{90\%\ Cd(OH)_2} (HC^*OO)_2Cd,Ni \xrightarrow[CuCr_2O_4]{H_2} C^*H_3OH$$

P. T. Adams, R. E. Selff and B. M. Tolbert, J. Am. Chem. Soc., *74*, 2416 (1952); Atomic Energy Commission Report, UCRL-1289; Nuc. Sci. Abstracts, *5*, 4405 (1951).

A. Procedure (Note 6)

A stirred mixture of 1.6 g. (18.3 mmoles) of potassium formate-C^{14}, 5 ml. of water and 10 ml. of wet-packed Dowex-50 cation exchange resin (Note 7) is filtered into a hydroxide slurry, the resin being washed with 7–8 portions of water (total 30 ml.). The alkali is prepared, in a 40-ml. centrifuge cone, by treating an aqueous solution of 0.30 g. of $Ni(NO_3)_2 \cdot 6\ H_2O$ and 2.08 g. of $CdCl_2 \cdot 2.5\ H_2O$ with sodium hydroxide, then washing the precipitate free of chloride with three portions of water. The salt mixture is evaporated to dryness on a steam-bath with an air stream, then transferred to a 115-ml. stainless steel hydrogenation bomb containing 1.5 g. of copper chromite (Note 8). The mixture is dried thoroughly by pumping on the bomb for 4–8 hours with high vacuum. The bomb is filled with electrolytic hydrogen to 3500 p.s.i., then heated at 240° with shaking for 9 hours and cooled; the hydrogen is exhausted through a spiral trap containing a sintered disc and cooled with liquid nitrogen (Figure X, 1). The product, collected in the trap by vacuum distillation at 80° for 5 hours, is a mixture of methanol-C^{14} and water; yield 85–90% (Note 9).

METHOD III

$$HC^*OOCH_3 \xrightarrow[CuCr_2O_4]{H_2} C^*H_3OH \xrightarrow{HI} C^*H_3I$$

D. B. Melville, J. R. Rachele and E. B. Keller, J. Biol. Chem., *169*, 419 (1947).

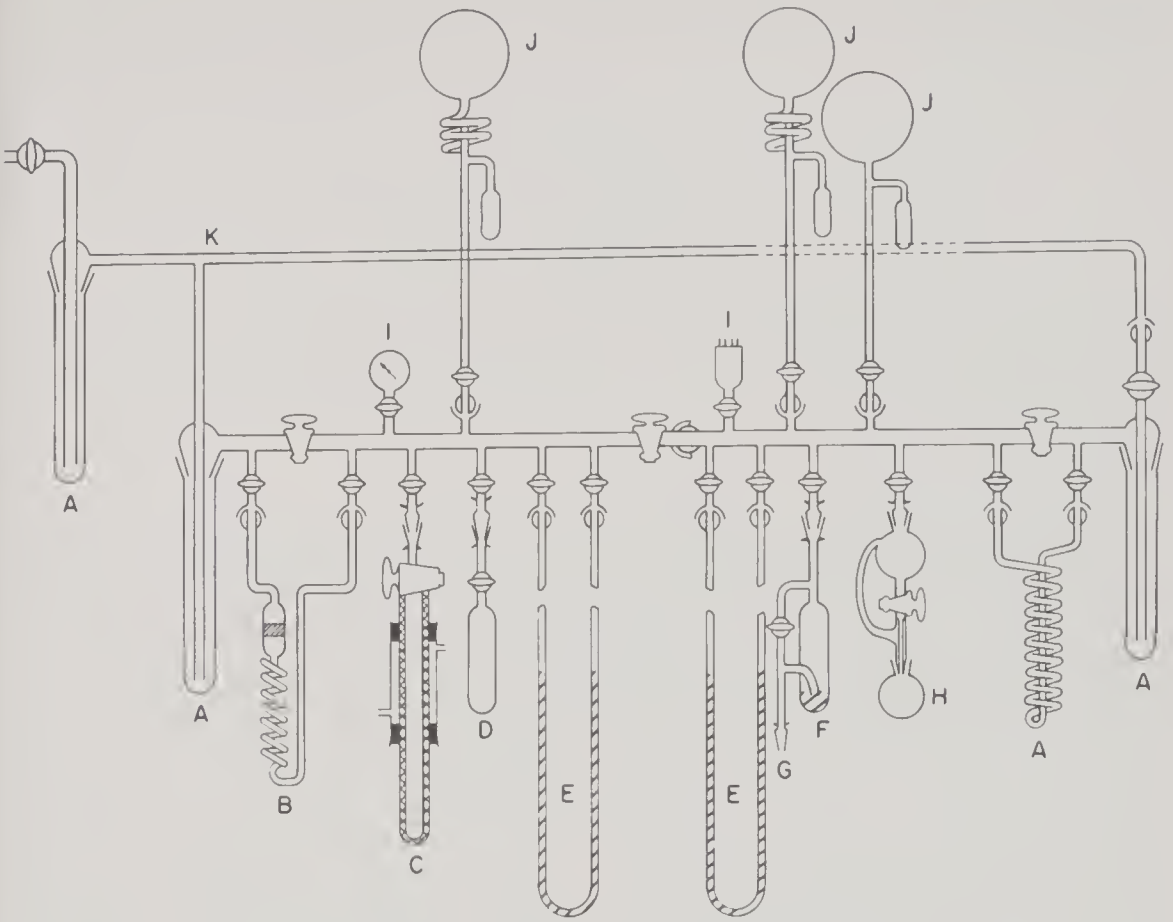


Fig. X, 1. Vacuum line for general organic syntheses (B. M. Tolbert). A, liquid nitrogen trap; B, spiral trap with sintered disc; C, iodination glass bomb; D, reaction or storage flask; E, mercury manometer; F, mercury bubbler with bypass; G, connection to hydrogenation bomb; H, carbon dioxide generator; I, vacuum gauge; J, gas storage and aliquoting bulbs with freezing arm; K, vacuum manifold.

A. Procedure

Methyl formate-C^{14} (0.2 ml.) is evaporated slowly at room temperature (within 1 hour) in a slow stream of hydrogen and is carried into a catalyst tube, maintained at 160° and containing copper chromite catalyst-on-silica chips.[14] The hydrogen and methanol-C^{14} formed are passed into 10 ml. of refluxing (135°), freshly distilled, constant-boiling hydriodic acid. The exhaust is passed through a suspension of red phosphorus in 4 ml. of water, then through a tube containing calcium chloride and a trap cooled with Dry Ice-cellosolve, the hydrogen sweep being continued for 30 minutes. The yield of methyl-C^{14} iodide is 0.37 ml. The method suffers an inherent 100% isotopic dilution, but the procedure handles volatile compounds without a vacuum system. The completeness of ester reduction and the purity of the methanol formed, in a trial run, are indicated by the refractive index, n_D^{10} 1.3292 (purified methanol n_D^{20}

1.3288). Indications are that the yield is nearly quantitative; see methyl-C^{14} iodide, C.

METHOD IV

$$C^*O_2 \xrightarrow[KCuAl_2O_3]{3H_2} C^*H_3OH$$

B. M. Tolbert, J. Am. Chem. Soc., *69*, 1529 (1947); M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon,* Wiley, New York, 1949, p. 167.

A. Procedure (Note 10)

Into an evacuated microbomb (Note 11), containing 5 g. of copper potassium alumina catalyst (Note 12), is distilled 25 mmoles of carbon-C^{14} dioxide by cooling with liquid nitrogen, (Figure X, 1). The bomb is closed and warmed to room temperature, hydrogen is added (Note 13) to 4000 p.s.i., and the bomb is heated to 285° for 6 hours (Note 14). The contents of the cooled bomb are discharged at the rate of 1–2 l. per minute through a spiral trap cooled with liquid nitrogen. The bomb is finally warmed and the contents of both it and the trap are vacuum-distilled into another trap on the line. The unreacted carbon-C^{14} dioxide (3–4%) is recovered by distillation through a spiral trap cooled with Dry Ice. The yield is 85–90% (Note 15), and the product contains appreciable water which cannot be separated readily.

B. Notes

1. The procedure is a modification of the method of Nystrom,[1] designed to eliminate the contamination of product with compounds arising from the splitting of solvent ethers,[2] which are difficult to remove at the semimicro level. Cox, Turner and Warne found that the original method, employing diethylene glycol diethyl ether and diethylene glycol monobutyl ether, lead to contamination with 4–7% ethanol and some butanol. Up to 25% formaldehyde is formed[1] in the presence of insufficient hydride.

Methanol-C^{14} has been prepared by Burns[3] by the original procedure; the hydrolysis reaction with the first few drops of diethylene glycol monobutyl ether was undesirably vigorous.

2. This semicyclic acetal is stable in alkaline media, has low volatility, will dissolve lithium aluminum hydride up to *M* at room temperature, and may be prepared by reaction of 3,4-dihydro-2*H*-pyran with tetrahydrofurfuryl alcohol.[4] The solution is prepared in an atmosphere of dry nitrogen by stirring the coarsely crushed hydride with the pure solvent and filtering the suspension through a sintered glass funnel. The clear solution is assayed by alcoholysis of an aliquot and measurement of the

evolved hydrogen—a procedure is described by Krynitsky.[5] High-purity lithium aluminum hydride is desirable, as it releases only a negligible amount of bound ether.[2]

3. Diagrams of suitable vacuum systems and apparatus are presented by Cox, Turner and Warne; also see C_1^{14}-ethanol, Figure X, 2.

4. The distillation is performed at 60 mm. with preparations at the 20-mmole scale, or larger.

5. The crude chemical yield is 100–105%, the impurities consisting of 2.5–4.5% water, 0.4% formaldehyde, 0.4% formals and less than 4% (each) solvent and tetrahydrofurfuryl alcohol. Formaldehyde-C^{14} is formed in considerable amount, if lithium aluminum hydride is not present in appreciable excess; see formaldehyde-C^{14}, Other Preparations (Weygand). The refractive index and density of the redistilled product indicate high purity. The product has been converted into very pure methyl-C^{14} iodide, in 77–85% yield based on carbon-C^{14} dioxide.

Methanol-C^{14} having a specific activity of 8–10 mc./mmole, after storage for 21 months in sealed ampoules, was found[6] to have undergone 19% decomposition from self-irradiation. The decomposition products were methane, hydrogen, glycol, glycerol and erythritol.

The preparation of methanol-C^{14}, essentially by the described procedure, has been described by Stutz.[7] A good yield was obtained only by stripping the stirred reaction mixture in several stages between 30 and 60° (20 μ), then redistilling at 0°. A 97.7% yield has been reported by Kögl.[8]

Nystrom[2] has modified the original procedure,[1] employing diethylcarbitol (rigorously purified over sodium) and butylcarbitol as solvent and alcohol interchange agent, respectively. Following decomposition of the excess lithium aluminum hydride, helium was passed through the mixture at 40° for 45 minutes to sweep out volatile contaminants (ether); then the alcoholysis was effected at 140° for 4 hours. The methanol-C^{14} was isolated from the distillate by four successive vacuum distillations at −45°; yield 92%, v.p. 29.0 mm. at 0°, n_D^{20} 1.330–1.332 (pure methanol n_D^{20} 1.3279; diethylcarbitol, n_D^{20} 1.6860). The molar specific activity was in agreement with that of the starting material. A diagram of the apparatus is presented by Nystrom.[2] The method has been described also by Neyman[9] with a diagram of the apparatus. Hydrolysis was accomplished with excess methanol.

6. The procedure is a modification of the general method of Richardson for reducing metal salts of fatty acids to alcohols; see 1-propanol-1-C^{14}.

7. The resin is prepared by treatment with 2 *N* hydrochloric acid and washed acid-free with water.

8. See 1-propanol-1-C^{14}, Note 5.

9. Mass spectrographic analysis shows that the product contains no ethanol or propanol. The absence of higher molecular weight analogues

was further indicated by conversion to methyl-C^{14} iodide and acetic-2-C^{14} acid with the expected equivalent weight.

10. The method is adapted from one described by Ipatieff.[10]

11. American Instrument Co., Silver Springs, Md. The free volume is 300 ml., and the system is evacuated to 30 μ.

12. The catalyst, containing 10% cupric oxide and 2% potassium hydroxide, is prepared by impregnating 8–12 mesh alumina (100 sq. meters surface per g.)[11] with copper nitrate, drying at 130°, saturating with potassium hydroxide solution, drying, igniting at 500° for 4 hours and reducing for 3 hours at 285° under 450 atmospheres of hydrogen. As the catalyst becomes unreliable after two runs, the efficiency of each new batch should be checked with a trial preparation.

13. Care must be exercised that the pressure of hydrogen on the supply side is always higher than that in the bomb. A booster pump may be required to fulfill this requirement. Reduction is incomplete below 3500 p.s.i.

14. The pressure reaches 7000 p.s.i.

15. The yield is based upon conversion to methyl iodide. Fractionation of the crude product from a trial run gave a product boiling at 65°, n_D^{22} 1.3332.

C. Other Preparations

The preparation of redistilled methanol-C^{13} in 90% yield at the 1-mole level, by the original procedure of Nystrom,[1] has been reported by Baddiley.[12] The preparation of methanol-C^{14} has been reported;[13] the diethyl carbitol was vacuum-distilled over lithium aluminum hydride below 60°.

Methanol-C^{11} has been prepared by Harman[14] by essentially Method III.

The preparation of methanol-C^{14}-1-H_3^2 by a modification of Method I has been reported by Rachele.[15] Carbon-C^{14} dioxide was reduced with lithium aluminum hydride-H_4^2, and the complex was decomposed with diethylene glycol monobutyl ether; see Nystrom.[1]

[1]R. F. Nystrom, W. H. Yanko and W. G. Brown, J. Am. Chem. Soc., *70*, 441 (1948).

[2]R. F. Nystrom, W. J. Skraba and R. G. Mansfield, Atomic Energy Commission Report, ORNL-395; Nuc Sci. abstracts, *4*, 5669 (1950); R. Ostwald, P. T. Adams and B. M. Tolbert, J. Am. Chem. Soc., *74*, 2425 (1952).

[3]G. R. Burns, J. Am. Chem. Soc., *77*, 6615 (1955).

[4]G. Forrest Woods and D. N. Dramer, J. Am. Chem. Soc., *69*, 2246 (1947).

[5]J. A. Krynitsky, J. E. Johnson and H. W. Carhart, Anal. Chem., *20*, 311 (1948).

[6]W. J. Skraba, J. G. Burr and D. N. Hess, J. Chem. Phys., *21*, 1296 (1953); R. M. Lemmon, Nucleonics, *11* (10), 44 (1953).

[7]R. E. Stutz, D. E. Atkinson and S. A. Gordon, Atomic Energy Commission Report ANL-4710; Nuc. Sci. Abstracts, *6*, 164 (1952).

[8]F. Kögl, P. Emmelot and D. H. W. den Boer, Ann., *589*, 1 (1954).

[9]M. B. Neyman and G. I. Feklisov, Doklady Akad. Nauk S.S.S.R., *87*, 605 (1952); Nuc. Sci. Abstracts, 7, 4095 (1953); AEC-tr-1828; F. I. Feklisov, Izvest. Akad. Nauk S.S.S.R., Otdel. Khim. Nauk, *1953*, 587; through Chem. Abstracts, *48*, 12666 (1954).

[10]V. N. Ipatieff and G. S. Monroe, J. Am. Chem. Soc., *67*, 2168 (1945).

[11]Aluminum Ore Co., America, Pittsburgh, Pa.

[12]J. Baddiley, G. Ehrensvärd, J. Johansson, L. Reio, E. Saluste and R. Stjernholm, J. Biol. Chem., *183*, 771 (1950); M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 168.

[13]G. R. Burns, J. Am. Chem. Soc., *77*, 6615 (1955).

[14]D. Harman, T. D. Stewart and S. Rubin, J. Am. Chem. Soc., *64*, 2293 (1942).

[15]J. R. Rachele, E. J. Kuchinskas, F. H. Kratzer and V. du Vigneaud, J. Biol. Chem., *215*, 593 (1955).

C_1^{14}-ETHANOL

METHOD I

$$CH_3C^*OOH \xrightarrow[10\%\ Ni(OH)_2]{90\%\ Cd(OH)_2} (CH_3C^*OO)_2Cd,\ Ni \xrightarrow[CuCr_2O_4]{H_2} CH_3C^*H_2OH \xrightarrow{P,I_2}$$

(a)

$$CH_3C^*H_2I$$

(b)

P. T. Adams, R. E. Selff and B. M. Tolbert, J. Am. Chem. Soc., *74*, 2416 (1952).

A. Procedure

(a) *Ethanol-1-C^{14}.* Sodium acetate-1-C^{14} (1.645 g.) is converted to ethanol-1-C^{14}, in 95% yield, according to the procedure for preparing 1-propanol-1-C^{14} or methanol-C^{14} (Method II). The method is reliable on a small scale, and no contaminating organic compounds are introduced.

(b) *Ethyl-1-C^{14} Iodide.* The ethanol-1-C^{14} is converted to the iodide, in 2.66 g. yield (85% based on sodium acetate), according to the procedure for preparing propyl-1-C^{14} iodide or methyl-C^{14} iodide (Method II); also see Other Preparations.

METHOD II

$$C^*H_3COCl \xrightarrow{LiAlH_4} C^*H_3CH_2OH$$

J. D. Cox and H. S. Turner, J. Chem. Soc., *1950*, 3176.

A. Procedure (Note 1)

Ethanol-2-C^{14}. To an outgassed, stirred solution of 13.3 mmoles of lithium aluminum hydride (100% excess) in 10.8 ml. of diethylene glycol diethyl ether at 0° (Note 2) is added 1.047 g. (13.3 mmoles) of acetyl-2-C^{14} chloride (Note 3) over a period of 15 minutes by vacuum distillation. The mixture is cooled to −78° and the system is re-evacuated through a trap at −196°, if the halide absorption is retarded by hydrogen evolution. The mixture is stirred at 0° (Note 4) for 2 hours, then alcoholyzed under nitrogen by the dropwise addition of 10 ml. (50% excess) of ethylene glycol monophenyl ether. The mixture is heated under reflux at 90° for 15 minutes and at 100° for 2 hours in a slow stream of nitrogen, the crude product being collected in the trap at −196° by distillation under conditions of partial reflux (Note 5). The cold trap is evacuated, and the product is distilled at 0° into a storage flask; yield 0.599 g. (n_D^{25} 1.3600, b.p. 78°, v.p. 12.2 mm. at 0°, Note 6), 96% based on sodium acetate or 91% on a radiochemical basis (Note 7).

METHOD III

$$C^*H_3COOC_2H_5 \xrightarrow[CuCr_2O_4]{H_2} C^*H_3CH_2OH \xrightarrow{PBr_3} C^*H_3CH_2Br$$

(a) (b)

B. M. Tolbert, F. Christenson, F. N. H. Chang and P. P. T. Sah, J. Org. Chem., *14*, 525 (1949).

A. Procedure

(a) *Ethanol-2-C^{14}* (Note 8). Ethyl acetate-2-C^{14} (8.5–12 mmoles) is vacuum-distilled into a 115-ml. hydrogenation bomb containing 5 g. of Adkins' copper chromite catalyst (see 1-propanol-1-C^{14}). The reactor is filled with hydrogen to 2500 p.s.i. at room temperature and heated for 10 hours at 250° with shaking. After cooling to room temperature, the hydrogen is exhausted through a spiral trap at −196° containing a fritted disc (Note 9). The contents of both the bomb and the spiral trap are vacuum-distilled into a liquid nitrogen-cooled trap. The product is a mixture of ethanol-2-C^{14}, water and a little C^{14}-ethyl ether (Note 10).

(b) *Ethyl-2-C^{14} Bromide.* The mixture of ethanol-2-C^{14} and water is vacuum-distilled into a small flask fitted with a sealed-on addition tube and low-temperature condenser cooled with Dry Ice-isopropanol (Note 2). A second low-temperature condenser is attached in series; then a large excess of phosphorus tribromide (2–3 ml.) is added, and the mixture is refluxed at 100° for 3 hours. The crude product is

vacuum-distilled into a tube containing 10 ml. of *N* sodium hydroxide, mixed at room temperature, then distilled with some water into another tube containing glass beads and 1 g. of phosphorus pentoxide. After drying at room temperature, the product is distilled into a third tube containing 5 ml. of concentrated sulfuric acid to remove ether and olefins, and then is distilled into a storage flask; yield 67–77% based on sodium acetate (Note 11).

B. Notes

1. The procedure is a modification of the isotopic method of Lorber.[1] The reduction of acetic acid with lithium aluminum hydride,[2] by the technique described, was unsatisfactory (40%), since the liberated hydrogen made diffusion very slow.

2. The vapor pressure of diethyl carbitol is 0.4 mm. at 20°. The solvent is pumped on at room temperature for a minute to remove dissolved gases, then cooled to −78°, and the pressure is reduced to 1 μ. A scale diagram of the vacuum apparatus is presented in Figure X, 2; also see Methanol-C^{14}, Method I.

3. The absorption of acetyl chloride (prepared by Method III) is quantitative but highly exothermic.

4. The specific activity of the product drops if the temperature is allowed to rise.

5. The reflux condenser is in the form of a cold finger.

6. The product contains 0.55% water,[3] 1.1% methanol,[4] less than 2% diethyl carbitol (on the basis of vapor pressure), and no detectable acetaldehyde.

7. There is no isotopic exchange with the solvent, diethylene glycol diethyl ether.

8. The method is the same as that for preparing methanol-C^{14} (Method III), but the procedure is quite different.

9. A diagram of the apparatus is presented by Tolbert.

10. The product from trial runs was dried over Drierite and distilled, b.p. 76–78°, n_D^{17} 1.3624.

11. The product from trial runs boiled at 34–39°, n_D^{18} 1.4326.

C. Other Preparations

Phenethyl-α-C^{14} alcohol is prepared[5] by Method II by reduction of phenylacetic-1-C^{14} acid. The carbinol may be assayed as the carbanilate. Degradation, by oxidation to benzoic acid (80%) in alkaline permanganate solution, shows that there is no isotopic rearrangement.

Ethanol-2-C^{14} has been prepared by Cox and Turner,[6] by reduction of ethyl acetate-2-C^{14} as described in Method II. As the absorption of the ester was slow (1.5 hours), the mixture was cooled to −78°

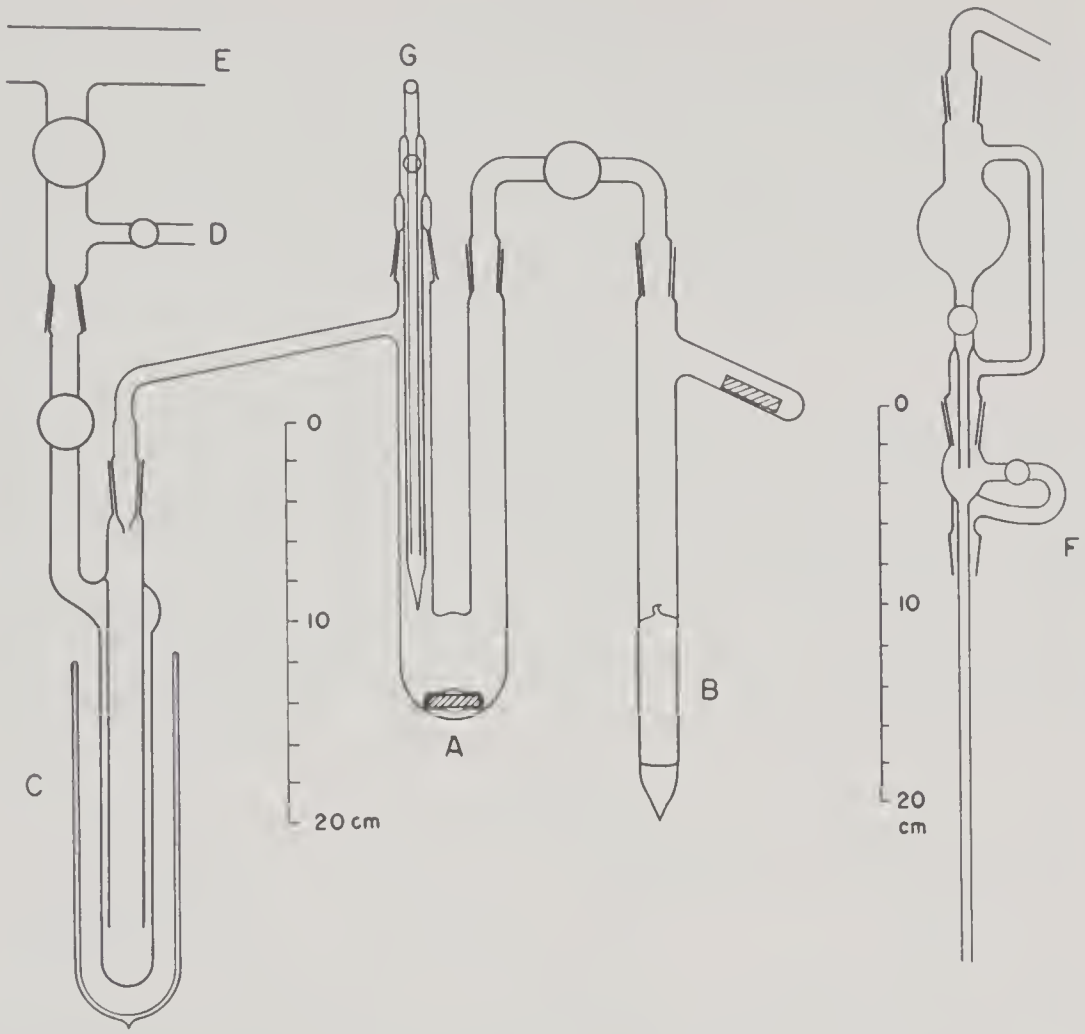


Fig. X, 2. Apparatus for reduction of acetyl chloride (J. D. Cox and H. S. Turner). A, reaction vessel with magnetic stirrer; B, break-seal tube with magnetic hammer; C, cold trap; D, nitrogen inlet; E, vacuum manifold; F, detached addition funnel; G, reflux condenser (dephlegmator).

and freed from permanent gases at intervals. The crude product (n_D^{25} 1.3608, v.p. 14.5 mm. at 0°) was treated with Raney nickel at room temperature (10 minutes) to remove hydrogen sulfide, which arose from the methyl sulfate used in preparation of the ester. The yield of purified product was 95.3% (corrected for 2.2% water) based on sodium acetate and 84% on a radiochemical basis. The quality (n_D^{25} 1.3604, v.p. 12.6 mm. at 0°) was inferior to that obtained by Method II. The use of a modification of this method has been reported by Pearce[7] (apparatus diagram) and Neyman[8] in preparing ethanol-1-C^{14}. The preparation of ethanol-2-C^{14} by reduction of the corresponding ethyl ester has been described by Neyman, and an apparatus diagram is given.

The preparation of ethanol-1-C^{14} by reduction of sodium acetate-1-C^{14} with lithium aluminum hydride has been reported by Chapman.[9]

The preparation of ethanol-1-C^{14} and ethanol-2-C^{14}, by the lithium aluminum hydride reduction of the corresponding C^{14}-acetic acids at room temperature in purified diethyl carbitol (see Method II), has been

reported by Roberts[10] and Ostwald.[11] Alcoholysis was achieved with butyl carbitol. The carbinols were converted[11] to ethyl-1-C^{14} and ethyl-2-C^{14} bromide by the described procedure. The apparent yield of 116% based on acetic acid is explained,[11] on the basis of activity data, by a 26% dilution of the intermediate with ethanol arising from scission of the solvent (see methanol-C^{14}, Method I). The similar preparation of ethanol-2-C^{13}-1-C^{14} has been reported by Ehrensvärd.[12] The preparation of ethanol-1-C^{14} has been reported by Finkelstein.[13] A degradation is described by Roberts.[10]

The preparation of ethanol-2-C^{14} by reduction of 1.0 g. of acetic-2-C^{14} acid with 0.634 g. of lithium aluminum hydride in 30 ml. of absolute ether is described by Roberts.[14] Hydrolysis was accomplished with 0.6 ml. of water, and the product was isolated by distillation with 20 ml. of carrier; radiochemical yield 56.6%.

The preparation of ethanol-1-C^{13} and ethanol-2-C^{13}, by Method III, has been reported by Sakami.[15] A similar preparation of ethanol-1-C^{14} has been described in detail by Andrianova;[16,8] yield 94% in trial runs, radiochemical yield 50–60% based on barium carbonate. Completeness of hydrogenation (29 hours, 250°, 445 atmospheres) was checked in a trial preparation by titration of excess alkali, following hydrolysis. Detailed directions are given[17] (2 diagrams) for the similar preparation of 1-propanol-1-C^{14} (yield 88%, b.p. 93–100°) by hydrogenation of propyl propionate-1-C^{14} at 250° and 405 atmospheres for 21 hours; and 2-methyl-1-propanol-1-C^{14} (radiochemical yield 75%, admixed with ethanol) by hydrogenation of ethyl 2-methylpropionate-1-C^{14} at 250° and 410 atmospheres for 27 hours in a rocking autoclave.

The preparations of ethanol-1-C^{13}, ethanol-2-C^{13} and ethanol-1-C^{13}-2-C^{14} have been reported by Lifson[18] and Lorber.[19,20] The corresponding butyl acetates were hydrogenated at 250° and 220 atmospheres over copper chromite. The butanol-alcohol mixtures were converted to the corresponding iodides with hydriodic acid and fractionated. The yields were not high.

Hydrogenation over a copper chromite catalyst (10% by weight) prepared under somewhat different conditions than those described by Adkins[21] has been reported[22] to give predominately hydrocarbons. Pentane-1-C^{14} was prepared by hydrogenation of ethyl valerate-1-C^{14} at 139 atmospheres and 280–285° for 3.5 hours; yield 85%, b.p. 35.5°, m.p. −130.5°, n_D^{10} 1.3578, radiochemical yield 30% based on barium carbonate. Pentane-3-C^{14} was prepared by hydrogenation of 3-pentanone-3-C^{14} at 116 atmospheres and 225–230° for 2 hours; yield 82%, radiochemical yield 30% based on barium carbonate.

Ethanol-2-C^{14} has been prepared by Burns[23] by a variation[24] of the oxo process.[25] The homologation reaction of methanol-C^{14} with carbon monoxide and hydrogen in the presence of dicobalt octacarbonyl[25] at

185° (3500–5000 p.s.i.) in a rocking autoclave yielded; ethanol-2-C^{14} (40%), acetic-2-C^{14} acid (14%) and 1-propanol-2,3-$C^{14}_{1/2}$ (5%). Di- reactions are given by Burns for working up the reaction mixture by hydrolyzing the acetals and esters, destroying the catalyst and fractionating the products with a Piros-Glover spinning-band column. Degradation studies indicate that the activities of C-2 and C-3 in propanol are about 50% of that of the starting material. This suggests the addition of carbon monoxide to some symmetrical intermediate from ethanol-2-C^{14}, such as ethylene-C^{14}_1.

Ethanol-2-C^{14} has been prepared by Neyman[8] (70% yield) by treating methylmagnesium-C^{14} iodide with gaseous formaldehyde, generated by pyrolytic decomposition of dry paraformaldehyde.

2-Propanol-1-C^{14} has been prepared by Kummer,[26] in a similar manner, by treating methylmagnesium-C^{14} iodide with acetaldehyde. The 2-bromopropane-1-C^{14}, formed from this carbinol with phosphorus tribromide, was converted[18] through the Grignard reagent to 2-methyl-1-propanol-3-C^{14} by treatment with formaldehyde. The constant-boiling water-alcohol mixtures were dehydrated with calcium carbide, fractionated, and employed in a study of the mechanism of the Fischer-Tropsch synthesis.

1-Propanol-1-C^{14} has been prepared by Roberts[27] (50% yield), by reducing propionic-1-C^{14} acid with lithium aluminum hydride.

2-Methyl-2-propanol-2-C^{14} has been prepared by Roberts[28] (59% yield) by reacting acetyl-1-C^{14} chloride with methylmagnesium chloride, then decomposing the complex with ammonium chloride.

The preparation of 2,3,3-trimethyl-2-butanol-1-C^{14}, by the reaction of methylmagnesium-C^{14} iodide with pinacolone, has been reported by Roberts.[29]

The preparation of ethyl-1-C^{14} bromide from the alcohol with hydrobromic acid has been reported by Zabin.[30]

The preparation of ethyl-1-C^{14} iodide by Method I has been described by Burtle.[31] To a mixture of 11.5 g. of 95% ethanol-1-C^{14} and 3.1 g. of red phosphorus, in a 200-ml. round-bottomed flask under a reflux condenser cooled with Dry Ice-acetone, is added 38.3 g. of iodine in nine portions at 10-minute intervals. The mixture is heated gently for 1 hour, stored overnight and steam-distilled. The colorless heavy distillate (33.8 g., 91.5%) is separated from water, washed with saturated sodium carbonate and water, dried over calcium chloride and distilled; yield 32.4 g. (87.6%), b.p. 70–73°.

The preparation of 2-iodopropane-1,3-C^{14}_2 (yield 75% based on barium carbonate-C^{14}) has been reported by Ostwald.[32] 2-Propanone-1,3-C^{14}_2, formed by pyrolysis of barium acetate-2-C^{14}, was subjected to high pressure hydrogenation over copper chromite, and the 2-propanol-1,3-C^{14}_2 was halogenated with iodine in the presence of phosphorus.

The preparation of 1-butanol-1-C^{14} at the small scale by reduction of butyric-1-C^{14} acid with lithium aluminum hydride has been reported by Gottlieb.[33] The alcohol was converted to butyl-1-C^{14} decanedioate and butyl-1-C^{14} dibutyl phosphate.

[1]V. Lorber, M. Cook and J. Meyer, J. Biol. Chem., *181*, 475 (1949); I. Siegel and V. Lorber, J. Biol. Chem., *189*, 571 (1951).

[2]*Organic Reactions*, Vol. VI, Wiley, New York, 1951, Chapter 10.

[3]K. Fischer, Angew. Chem., *48*, 394 (1935); G. B. Levy, J. J. Murtaugh and M. Rosenblatt, Ind. Eng. Chem., Anal. Ed., *17*, 193 (1945).

[4]C. Simmonds, Analyst, *37*, 16 (1912).

[5]C. C. Lee and J. W. T. Spinks, Can. J. Chem., *32*, 1005 (1954).

[6]J. D. Cox and H. S. Turner, J. Chem. Soc., *1950*, 3176.

[7]G. W. Pearce and J. A. Jensen, Science, *118*, 45 (1953); J. Agr. Food Chem., *1*, 776 (1953).

[8]M. B. Neyman and G. I. Feklisov, Doklady Akad. Nauk S.S.S.R., *87*, 605 (1952); Chem. Abstracts, *48*, 114 (1954); Nuc. Sci. Abstracts, 7, 4095 (1953); AEC-tr-1828; G. I. Feklisov, Izvest. Akad. Nauk S.S.S.R., Otdel. Khim. Nauk, *1953*, 587; Chem. Abstracts, *48*, 12666 (1954).

[9]D. D. Chapman, G. W. Brown, Jr., I. L. Chaikoff, W. G. Dauben and N. O. Fansah, Cancer Research, *14*, 372 (1954).

[10]J. D. Roberts and J. A. Yancey, J. Am. Chem. Soc., *74*, 5943 (1952).

[11]R. Ostwald, P. T. Adams and B. M. Tolbert, J. Am. Chem. Soc., *74*, 2425 (1952).

[12]G. Ehrensvärd, L. Reio, E. Saluste and R. Stjernholm, J. Biol. Chem., *189*, 93 (1951).

[13]A. Finkelstein and W. A. Noyes, Jr., Discussions Faraday Soc., *14*, 81 (1953).

[14]R. M. Roberts, G. A. Ropp and O. K. Neville, J. Am. Chem. Soc., *77*, 1764 (1955).

[15]M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 202.

[16]T. I. Andrianova, E. A. Andreev and O. M. Sokolova, Doklady Akad. Nauk S.S.S.R., *88*, 677 (1953); Chem. Abstracts, *48*, 2565 (1954); Nuc. Sci. Abstracts, 7, 3057 (1953); AEC-tr-1827.

[17]T. I. Andrianova and E. A. Andreev, Doklady Akad. Nauk S.S.S.R., *86*, 1105 (1952); AEC-tr-1824; Nuc. Sci. Abstracts, 7, 4100 (1953).

[18]N. Lifson, V. Lorber, W. Sakami and H. G. Wood, J. Biol. Chem., *176*, 1263 (1948).

[19]V. Lorber, N. Lifson, H. G. Wood, W. Sakami and W. W. Shreeve, J. Biol. Chem., *183*, 517 (1950).

[20]V. Lorber, N. Lifson, W. Sakami and H. G. Wood, J. Biol. Chem., *183*, 531 (1950).

[21]H. Adkins and R. Connor, J. Am. Chem. Soc., *53*, 1091 (1931).

[22]A. F. Lukovnikov, M. B. Neyman, A. A. Bag, L. M. Rodionova, I. S. Samoukina and N. V. Bliznyak, Doklady Akad. Nauk S.S.S.R., *88*, 297 (1953); Nuc. Sci. Abstracts, 7, 3054 (1953); AEC-tr-1829.

[23]G. R. Burns, J. Am. Chem. Soc., 77, 6615 (1955).

[24]I. Wender, R. A. Friedel and M. Orchin, Science, *113*, 206 (1951); I. Wender, R. Levine and M. Orchin, J. Am. Chem. Soc., *71*, 4160 (1949); *72*, 4375 (1950); K. H. Ziesecke, Brennstoff Chem., *33*, 385 (1952); K. B. Kulkarni, Bombay Technologist, *2*, 37 (1952); H. Hoog, Chem. Weekblad, *49*, 614 (1953).

[25]I. Wender, H. Greenfield and M. Orchin, J. Am. Chem. Soc., 73, 2656 (1951); I. Wender, M. Orchin and H. H. Storch, J. Am. Chem. Soc., 72, 4842 (1950).
[26]J. T. Kummer and P. H. Emmett, J. Am. Chem. Soc., 75, 5177 (1953).
[27]J. D. Roberts and M. Halmann, J. Am. Chem. Soc., 75, 5759 (1953).
[28]J. D. Roberts, Nucleonics, 7 (3), 59 (1950).
[29]J. D. Roberts, Atomic Energy Commission Report, NP-3391; Nuc. Sci. Abstracts, 5, 6708 (1951).
[30]I. Zabin and K. Bloch, J. Biol. Chem., *192*, 261 (1951).
[31]J. C. Burtle and W. N. Turek, J. Am. Chem. Soc., 76, 2498 (1954).
[32]R. Ostwald, Atomic Energy Commission Report, UCRL-2154; Nuc. Sci. Abstracts, 7, 3404 (1953).
[33]H. Gottlieb, J. Sorden and K. Guenther, Abstracts 121st Meeting Am. Chem. Soc., 91K (1952).

ETHANOL-C_2^{14}

$$BaC^*O_3 \xrightarrow{Ba} BaC^*_2 \xrightarrow{H_2O} HC^* \equiv C^*H \xrightarrow[HCl]{CrCl_2} C^*H_2 = C^*H_2 \xrightarrow{HOCl}$$

(a) (b)

$$ClC^*H_2C^*H_2OH \xrightarrow{KOH} C^*H_2C^*H_2 \xrightarrow{LiAlH_4} C^*H_3C^*H_2OH$$

(c) (d) (e)

J. D. Cox and R. J. Warne, J. Chem. Soc., *1951*, 1893.

A. Procedure

(a) *Acetylene-*C_2^{14} (Note 1). Barium carbonate-C^{14} (0.2988 g., 1.515 mmoles) is mixed with 1 g. of finely shredded barium metal in a centrifuge tube, and the mixture is covered with a like amount of the metal. A slow stream of dry, carbon dioxide-free argon is passed through the tube (Note 2), which is then heated in a Bunsen flame until the reaction mixture becomes incandescent and sets to a black mass, which is cooled in a dessicator. The tip of the tube containing the reaction mixture is quickly broken off inside a dry, two-necked, round-bottomed flask, which is fitted immediately with an addition tube and reflux condenser attached to traps cooled with Dry Ice-methanol and with liquid nitrogen (Note 3). After air is swept from the system with hydrogen, 30 ml. of water is added slowly to the barium carbide-C_2^{14}. The resulting solution is brought to a boil during 20 minutes (Note 4), and refluxing is continued for 20 minutes. The trap containing the crude product is attached to a vacuum system, and the product is distilled into a flask containing activated charcoal

pellets (Note 5) and cooled with a Dry Ice-bath (Note 6). Acetylene then is desorbed from the charcoal at 0° and vacuum-distilled into a receiver cooled with liquid nitrogen. Pressure-volume measurements indicate an apparent yield of 1.03 mmoles (137%); the radiochemical yield is 97%.

(b) *Ethylene-C_2^{14}* (Note 7). Acetylene-C_2^{14} (4.98 mmoles) is distilled into an evacuated flask containing 5 ml. of hydrochloric acid and the chromous chloride[1] prepared from 11 g. of chromic chloride hexahydrate. The flask is closed off, removed from the system and shaken at room temperature for 6 hours. The reaction mixture is frozen again in liquid nitrogen, and the pressure is reduced to 1 μ. The product is collected by two fractional vacuum distillations at −78°, the receivers being cooled at −196°; yield 95% (Note 8).

(c) *2-Chloroethanol-C_2^{14}*, (*Ethylene-C_2^{14} Chlorohydrin*). A flask containing 5 ml. of freshly prepared 1.8 *M* hypochlorous acid[2] solution (Note 9) is cooled to −78° and evacuated to remove traces of chlorine. Ethylene-C_2^{14} (4.07 mmoles) is added by distillation with liquid nitrogen, and the flask is closed off and shaken for 2 hours at room temperature. The reaction mixture is frozen in liquid nitrogen and pumped down. Traces of unreacted ethylene-C_2^{14} and chlorine are removed by vacuum distillation at −78°.

(d) *Ethylene-C_2^{14} Oxide*. To the still-frozen solution of ethylene-C_2^{14} chlorohydrin are added quickly 6 g. of potassium hydroxide pellets and a magnetic stirring bar. The system, including a trap at −78° and another (isolated by a stopcock) at −195°, is re-evacuated. As the reaction mixture is stirred and allowed to melt, a vigorous evolution of ethylene-C_2^{14} oxide takes place, and the product is collected in the trap at −196° by periodic opening of the stopcock. The small amounts of ethylene-C_2^{14} chlorohydrin condensed at −78° are distilled back into the alkaline mixture until reaction is complete; yield 3.50 mmoles, 84.2% (Note 10).

(e) *Ethanol-C_2^{14}*. Ethylene-C_2^{14} oxide (15.95 mmoles) is reacted at 0° with a 250%-excess of lithium aluminum hydride in tetrahydrofurfuryloxytetrahydropyran (0.71 *M*, 20 ml.), gas absorption being complete within 1 hour. Tetrahydrofurfuryl alcohol (15 ml.) is added, and the product is swept by a stream of hydrogen from the reaction mixture at 100° (5 hours) under conditions of partial reflux (cold finger). The crude distillate, collected in a trap at −196°, is purified by vacuum distillation at room temperature with the aid of liquid nitrogen; yield 0.746 g. (Note 11). The radiochemical yield is 93.3%.

B. Notes

1. The preparation at the 2 to 20-micromole level, in better than 90% yield by refinement of this procedure, has been described in detail by Arrol.[3]

2. The tube is warmed to remove traces of moisture.

3. The last trap is attached to guard tubes containing magnesium perchlorate and soda lime, and to a bubbler containing ammoniacal cuprous chloride solution.

4. When the solution is rapidly brought to boiling, the evolution of acetylene tends to become so violent that some gas escapes the liquid nitrogen-cooled trap. Any acetylene precipitated in the bubbler as cuprous acetylide, however, may be recovered by treatment with saturated potassium cyanide in a vacuum system.

5. The charcoal is heated at 200° under vacuum, prior to use.

6. Since acetylene reacts with mercury mirrors, it is necessary to isolate manometers by means of stopcocks whenever any part of the system is cooled. A titration of acetylene is described by Willstätter.[4,18]

7. The preparation, in 96–98% yield at the micromole level, by the described method has been reported by Arrol and Glascock.[1]

8. Yields in trial runs have been as high as 99.8%. The product gives no color with ammoniacal cuprous chloride solution and completely dissolves in mercuric sulfate-sulfuric acid solution.[6] Traces of acetylene may be removed by shaking with alkaline mercuric cyanide solution.[7]

9. More dilute, or impure, acid solutions give inferior yields of ethylene chlorohydrin. The method is a modification of one described by Gomberg.[8]

10. In calculating the yield of product, corrections are made for the deviation from the ideal gas laws shown by ethylene oxide.[9]

11. Analysis[10] shows the presence of about 3% methanol. Methods for degrading ethanol are described by Koshland[11] and Roberts.[12]

C. Other Preparations

The preparation of acetylene-C_2^{14}, by essentially the method described, has been reported by Beamer[13] (91% acetylene, 3% ethylene and 2.5% ethane), Monat[14] (93.7–98.8% yield, determined by silver nitrate titration), Nystrom[8] (96% radiochemical yield, ammonia- and phosphine-free), Walz,[15] Roberts,[16] Inhoffen[17] (94–96% yield at the 0.5-mmole level), and Pichat[18] (95–97% radiochemical yield, 105–115% chemical yield—due to barium carbonate in the barium).

The preparation of acetylene-C_2^{14} (-C_2^{13} or -C_2^{11}), by the similar reduction of barium carbonate-C* with magnesium, has been described by Cramer,[19] Sakami[20] (75% yield at the 51-mmole level, diagram), Vennesland[21] (50–70% yield, diagram), Wood,[22] Abrams (75–85% yield at the 1-mmole level, determined by titration;[4] see sodium acetate-C_2^{14}), Reitz[23] (diagram and details), Ferguson[24] (purified by three vacuum distillations at Dry Ice temperature into a trap at liquid nitrogen temperature), and Keyer[25] (up to 85%, 2 diagrams, fresh filings, 2-g. reaction tablets heated

at 700–950° for 30–40 minutes in a current of hydrogen in a stainless steel pipe, no need for rapid cooling).

The preparation of ethylene-C_2^{14}, by the described procedure, has been reported by Walz.[15] Arrol and Glascock[5] have found that the catalytic reduction of acetylene was possible at the micro-scale (75% yield) by using palladium-black at room temperature, but the catalyst was easily poisoned and the method not to be preferred.

Ethylene-C_2^{13} has been prepared in a roundabout manner by Wood,[22] through the following sequence of intermediates: acetaldehyde-C_2^{13}, acetic-C_2^{13} acid, acetyl-C_2^{13} chloride, ethanol-C_2^{13}, ethyl-C_2^{13} iodide, ethyl-C_2^{13}-trimethylammonium iodide and ethyl-C_2^{13}-trimethylammonium hydroxide (see 1,3-butadiene-2,3-C_2^{14}). Ethylene-C_1^{13} has been prepared in the same manner from acetic-1-C^{13} acid, acetyl-1-C^{13} chloride, ethanol-1-C^{13}, ethyl-1-C^{13} iodide, ethyl-1-C^{13}-trimethylammonium iodide and ethyl-1-C^{13}-trimethylammonium hydroxide. Also see ethylene-C_1^{14}. The C^{13} isotope effect in the thermodecomposition of C_1^{13}-ethyl bromide (350–450°) is described by Friedman.[26]

2-Bromoethanol-C_2^{14} has been prepared by Walz[15] (90% yield), by reacting equimolar amounts of ethylene-C_2^{14} and *N*-bromoacetamide in aqueous solution in the presence of sulfuric acid catalyst.

Ethylene-C_2^{14} oxide has been prepared by Tomisek[27] (30% yield), by reacting ethylene-C_2^{14} with perbenzoic acid in tetrachloroethane in the presence of iodine catalyst. Most olefin oxides are obtained in high yield by this method.[28] Pilgeram[29] has prepared ethylene-C_2^{14} oxide, essentially by the described method, in 55–70% yield based on barium carbonate (see ethanolamine-C_2^{14} hydrochloride), while Pichat[18] has reported a yield of 73% based on acetylene.

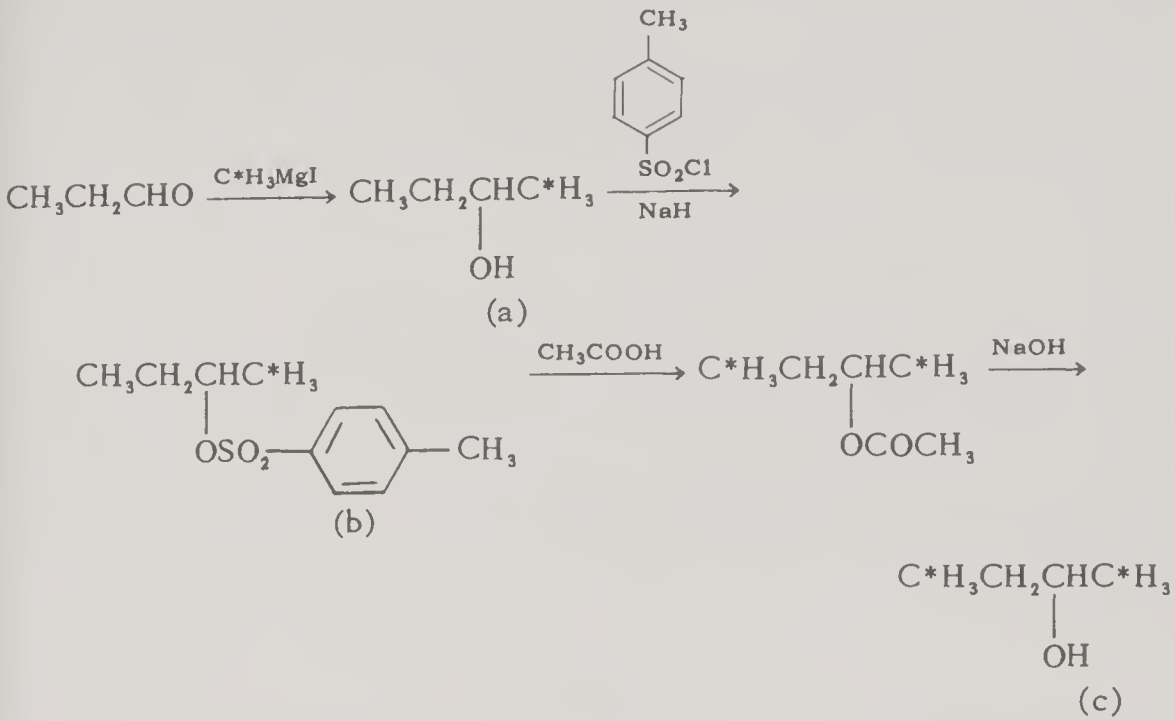
Ethanol-$C_{1/2}^{14}$ has been prepared by Roberts,[12] in 38–45% yield (b.p. 77–78°), by reacting ethylamine-1-C^{14} perchlorate with nitrous acid[30] (see phenethyl-α,β-$C_{1/2}^{14}$ alcohol and 1-propanol-1,2-$C_{1/2}^{14}$). The product was assayed as the 3,5-dinitrobenzoate, m.p. 92.5–92.8°. Degradation of the carbinol to iodoform, with sodium hypoiodite, showed that 1.5 ± 0.1% of the activity is located at C-2 as a result of rearrangement.[31] Experiment has shown that this rearrangement is not due to hydration of the by-product ethylene-C_1^{14} (isolated as the dibromide in 5.2% yield) during the decomposition of the diazonium salt. Ethylene-C_1^{14} was prepared by debromination with zinc.

The preparation of acetylene-C_2^{14} in 65% yield from barium carbonate-C^{14} by the method of Maquenne has been reported by Johnson.[32] The product was used in a study of the separate sorption and oxidation processes of acetylene on supported silver nitrate, carbon-C^{14} dioxide being formed above 149° after a definite amount of sorption (depending on the temperature) has taken place.

The preparation of ethanol-C_2^{14} by hydrogenation of acetaldehyde-C_2^{14} over Raney nickel at 170° has been reported by Isagulyants;[33] yield 60–75% based on acetylene.

[1]M. R. Hatfield, *Inorganic Syntheses*, Vol. III, McGraw, New York, 1950, p. 148.
[2]S. Reformatzky, J. prakt. Chem., *40*, 396 (1889).
[3]A. J. Arrol and R. Glascock, Nature, *159*, 810 (1947); J. Chem. Soc., *1948*, 1534.
[4]R. Willstätter and E. Maschmann, Ber., *53*, 939 (1920).
[5]W. J. Arrol and R. Glascock, J. Chem. Soc., *1949*, S335.
[6]A. W. Francis and S. J. Lukasiewicz, Anal. Chem., *17*, 703 (1945).
[7]R. Nystrom, Y. Loo, K. Mann and J. Allen, Nucleonics, 7 (3), 51 (1950).
[8]M. Gomberg, J. Am. Chem. Soc., *41*, 1414 (1919).
[9]O. Maass and E. H. Boomer, J. Am. Chem. Soc., *44*, 1709 (1922).
[10]C. Simmonds, Analyst, *37*, 16 (1912).
[11]D. E. Koshland, Jr. and F. H. Westheimer, J. Am. Chem. Soc., *72*, 3383 (1950).
[12]J. D. Roberts and J. A. Yancey, J. Am. Chem. Soc., *74*, 5943 (1952).
[13]W. H. Beamer, Nucleonics, 7 (3), 51 (1950).
[14]S. Monat, C. Robbins and A. R. Ronzio, Atomic Energy Commission Report, AECU-672; Nuc. Sci. Abstracts, *4*, 1407 (1950).
[15]D. E. Walz, M. Fields and J. A. Gibbs, J. Am. Chem. Soc., *73*, 2968 (1951).
[16]J. D. Roberts, C. C. Lee and W. H. Saunders, Jr., J. Am. Chem. Soc., *76*, 4501 (1954).
[17]H. H. Inhoffen, U. Schwieter, C. O. Chichester and G. Mackinney, J. Am. Chem. Soc., 77, 1053 (1955).
[18]L. Pichat, C. Baret, M. Audinot, M. Herbert and J. Lambin, *Radioisotope Conference, 1954*, Vol. I, Academic Press, New York, 1954, p. 245
[19]R. D. Cramer and G. B. Kistiakowsky, J. Biol. Chem., *137*, 549 (1941).
[20]W. Sakami, W. E. Evans and S. Gurin, J. Am. Chem. Soc., *69*, 1110 (1947); M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert, P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 205.
[21]B. Vennesland, A. K. Solomon, J. M. Buchanan, R. D. Cramer and A. B. Hastings, J. Biol. Chem., *142*, 371 (1942).
[22]H. G. Wood, J. Biol. Chem., *194*, 905 (1952).
[23]H. C. Reitz, F. J. Carleton and J. E. Christian, J. Am. Pharm. Assoc., Sci. Ed., *42*, 85 (1953).
[24]R. F. Ferguson, J. Chem. Phys., *23*, 2085 (1955).
[25]N. P. Keyer, B. V. Klimenok and G. V. Isagulyants, Doklady Akad. Nauk S.S.S.R., *85*, 1029 (1952); Nuc. Sci. Abstracts, 7, 457 (1953); AEC-tr-1825.
[26]H. L. Friedman, R. B. Bernstein and H. E. Gunning, J. Chem. Phys., *23*, 109 (1955).
[27]A. J. Tomisek and H. R. Mahler, J. Am. Chem. Soc., *73*, 4685 (1951).
[28]D. Swern, Chem. Revs., *45*, 16 (1949).
[29]L. O. Pilgeram, E. M. Gal, E. N. Sassenrath and D. M. Greenberg, J. Biol. Chem., *204*, 367 (1953).
[30]F. C. Whitmore and R. S. Thorpe, J. Am. Chem. Soc., *63*, 1118 (1941).
[31]F. C. Whitmore, J. Am. Chem. Soc., *54*, 3274 (1932).
[32]W. T. M. Johnson and K. A. Krieger, J. Am. Chem. Soc., *76*, 4782 (1954); L. Maquenne, Bull. soc. chim., *8*, 773 (1892).
[33]G. V. Isagulyants and O. A. Golovina, Doklady Akad. Nauk S.S.S.R., *93*, 659 (1953).

2-BUTANOL-1,4-$C^{14}_{1/2}$



J. D. Roberts, W. Bennett, R. E. McMahon and E. W. Holroyd, Jr., J. Am. Chem. Soc., 74, 4283 (1952).

A. Procedure

(a) *2-Butanol-1-C^{14}*. To a stirred, Dry Ice-cooled solution of 300 ml. of ether and the methylmagnesium-C^{14} iodide, prepared from 26 g. (0.18 mole) of methyl-C^{14} iodide and 5 g. of magnesium turnings, is added slowly 10.2 g. (0.18 mole) of freshly distilled propionaldehyde. The mixture is warmed to 0° and hydrolyzed by the dropwise addition of 30 ml. of saturated ammonium chloride solution. The ether phase is separated, and the salt cake is washed with three 100-ml. portions of ether. The combined extract is dried over calcium sulfate and concentrated by distillation of the solvent through a glass helix-packed column and the residue is fractionated through an efficient center-tube column;[1] yield 6.89 g. (53%), b.p. 100.3–100.5° (Note 1). Degradation studies show that there is no isotopic rearrangement (Note 2).

(b) *1-Methyl-C^{14}-propyl* p-*Toluenesulfonate* (Note 3). A solution of 4.9 g. (66 mmoles) of 2-butanol-1-C^{14} in 20 ml. of ether is added to a stirred mixture of 2.0 g. (86 mmoles) of sodium hydride and 50 ml. of absolute ether in a 500-ml. three-necked flask under nitrogen. The mixture is refluxed for 6 hours and stored overnight. A solution of 11.4 g. (60 mmoles) of *p*-toluenesulfonyl chloride is added dropwise; then the mixture is refluxed for 30 minutes and hydrolyzed by the slow addition of 30 ml. of water. The ether phase is separated, washed with 10% sodium

carbonate solution and dried over a mixture of potassium carbonate-magnesium sulfate. The mixture is concentrated under reduced pressure, transferred to a molecular still containing 1 g. of anhydrous potassium carbonate and distilled with a bath at 59–61° (3 μ). The yield of chloride-free product is 9.9 g. (72%).

(c) *2-Butanol-1,4-$C^{14}_{1/2}$* (Note 4). 1-Methyl-C^{14}-propyl *p*-toluenesulfonate (2.23 g., 10 mmoles) is heated at 65° for 24 hours with 100 ml. of acetic acid containing 0.100 *M* potassium acetate, 0.89 *M* potassium *p*-toluenesulfonate and 1% acetic anhydride. The mixture is made basic with 0.2 mole of strong potassium hydroxide solution, sufficient water is added to bring about solution, and the mixture is heated for 6 hours on a steam-bath. Carrier 2-butanol (5.0 g.) is added, and the product is continuously extracted into ether for 6 hours and worked up as above; yield 1.74 g., b.p. 100.0–101.0°. Degradation studies (Note 2) indicate that 9% of the specific activity is located at C-4 as a result of rearrangement (Note 5).

B. Notes

1. The forerun is 0.5 g., b.p. 98.5–100.3°. The product is assayed as the 1-methylpropyl carbanilate.

2. Directions are given by Roberts for oxidizing 2-butanol-1-C^{14} with excess sodium hypobromite, forming carbon-C^{14} tetrabromide (C-1) which is more readily purified than is the analogous iodoform-C^{14}.

3. The procedure is adapted from the method described by Roberts.[2]

4. The solvolysis reaction also has been run in aqueous-acetone (25–75% v/v) solution with 3% (or less) rearrangement (yield 77%), thus eliminating the possibility that the rearrangement had occurred in the preparation of the tosylate; also see Roberts.[2]

5. Experiment with isotopic butene, nonisotopic *s*-butyl acetate and the acetic acid mixture shows that the rearrangement cannot involve dehydration and addition of acetic acid to the olefin, for the ester does not gain activity. The % rearrangement = (activity of 2-butanol − activity of carbon tetrabromide) ÷ activity of 2-butanol.

Isotopic butenes are prepared by heating 5.0 g. of 2-butanol-1-C^{14} with 15 ml. of water and 20 ml. of concentrated sulfuric acid.[3] The hydrocarbon is purified by passage through 10% sodium hydroxide solution, 50% sulfuric acid and Drierite, then condensed at −70°; yield 2.0 g., 50%.

C. Other Preparations

In a similar manner from acetaldehyde is prepared[4] 2-propanol-1-C^{14}, which is converted to 2-bromopropane-1-C^{14} by the action of phosphorus tribromide. The reaction of the Grignard reagent with formaldehyde gas

gives 2-methylpropanol-3-C^{14}; the constant-boiling alcohol-water mixtures are dehydrated with calcium carbide, and the product is distilled.

[1]E. A. Naragon and C. J. Lewis, Ind. Eng. Chem., Anal. Ed., *18*, 448 (1946).
[2]J. D. Roberts and V. C. Chambers, J. Am. Chem. Soc., *73*, 5034 (1951).
[3]C. E. Wilson and H. J. Lucas, J. Am. Chem. Soc., *58*, 2396 (1936).
[4]J. T. Kummer and P. H. Emmett, J. Am. Chem. Soc., *75*, 5177 (1953).

PHENETHYL-α,β-$C^{14}_{1/2}$ ALCOHOL

$$C_6H_5CH_2C^*OOH \xrightarrow{SOCl_2} C_6H_5CH_2C^*OCl \xrightarrow{NH_3} \underset{(a)}{C_6H_5CH_2C^*ONH_2} \xrightarrow{LiAlH_4}$$

$$\underset{(b)}{C_6H_5CH_2C^*H_2NH_2} \xrightarrow{HONO} \underset{(c)}{C_6H_5C^*H_2C^*H_2OH}$$

C. C. Lee and J. W. T. Spinks, Can. J. Chem., *31*, 761 (1953).

A. Procedure

(a) *2-Phenylacetamide-1-C^{14}.* A mixture of 0.400 g. of phenylacetic-1-C^{14} acid and 2 ml. of purified[1] thionyl chloride is refluxed for 30 minutes. The mixture is evaporated to dryness under reduced pressure, and the residue is dissolved in absolute ether. Ammonia is bubbled into the solution, and the precipitated product and ammonium chloride are collected, washed with water to remove the ammonium salt and dried; yield 0.288 g. (75%), m.p. 155–157°.

(b) *Phenethylamine-α-C^{14},* (*2-Phenylethylamine-1-C^{14}*) (Note 1). To a solution of 40 ml. of absolute ether and 0.40 g. of lithium aluminum hydride, in a three-necked flask equipped with reflux condenser, sealed-stirrer and dropping funnel, is added 0.250 g. of 2-phenylacetamide-1-C^{14} in 250 ml. of ether. The mixture is stirred for 6 hours, then decomposed by the addition of an excess of 10% sodium hydroxide solution, and the aqueous layer is separated and extracted with ether. To the combined ether solution is added 5 g. of carrier phenethylamine; the solution is dried over sodium sulfate and concentrated with the use of an efficient fractionating column, and the residue is distilled. The yield is 4.840 g., b.p. 195–198°, and the radiochemical yield is 61.5% (Note 2). Degradation studies indicate that the isotope is located only at C-α (Note 3).

(c) *Phenethyl-α,β-$C^{14}_{1/2}$ Alcohol,* (*2-Phenylethanol-$C^{14}_{1/2}$*). To a stirred, ice-cooled solution of 3.63 g. (30 mmoles) of phenethylamine-α-C^{14} in 50% acetic acid (Note 4) (just acid to bromophenol blue) is added slowly a solution of 4.14 g. (60 mmoles) of sodium nitrite in 10 ml. of water and

sufficient acid to keep the medium acidic. The mixture is stirred for 1 hour at room temperature, during the further addition of 2.07 g. (30 mmoles) of solid sodium nitrite and the requisite acid. The mixture is heated for 10 minutes on a steam-bath, cooled and extracted with ether. The extract is washed with dilute acid, alkali and water, then dried over sodium sulfate, concentrated and distilled; yield 76–78%, b.p. 215–218° (Note 5). Degradation studies indicate that 20.5–22.5% of the isotope is found at C-β, due to rearrangement (Note 6).

B. Notes

1. The procedure is a modification of the method of Uffer[2] for reducing an amide to an amine of the same number of carbon atoms. Using a small amount of amide in a large volume of ether overcomes the difficulties due to the slight ether solubility of the starting material. The reaction, requiring 30–40 hours, gives a yield of 50% by the original procedure at the 5–10 g. scale.

2. The amine is assayed as 1-phenethyl-α-C^{14}-3-phenyl-2-thiourea, m.p. 135°, which is prepared by mixing equal amounts of the amine and phenyl isocyanate in a test tube, with warming if reaction is not immediate. The mixture is chilled in an ice-bath, then powdered, washed with ligroin and 50% ethanol and finally recrystallized from alcohol.

3. Directions are given by Lee for oxidizing the amine with alkaline permanganate, forming nonisotopic benzoic acid in 84% yield.

4. The product yield is 61–66% in 10% hydrochloric acid, and 50–54% in 10% perchloric acid medium. In anhydrous acetic acid there is formed phenethyl-α,β-$C^{14}_{1/2}$ acetate (69–73%, n_D^{25} 1.5064, b.p. 218–220°) which does not react with phenyl isocyanate. The ester is converted to phenethyl-α, β-$C^{14}_{1/2}$ alcohol in 68% yield, n_D^{25} 1.5321, by treatment with lithium aluminum hydride, or it is oxidized directly; see Note 6.

5. Vacuum distillation has little effect on the yield. The product is assayed as the phenylurethan, which is prepared by warming together equal weights (0.5 g.) of phenethyl-α,β-$C^{14}_{1/2}$ alcohol and phenyl isocyanate with a few drops of pyridine on a steam-bath for 15 minutes. The mixture is cooled in an ice-bath and stirred with a few ml. of petroleum ether. The crystals are filtered off, washed thoroughly with petroleum ether and collected in 90% yield. Recrystallization from chloroform-petroleum ether gives pure phenethyl-α,β-$C^{14}_{1/2}$ carbanilate, m.p. 79–80°. Roberts[3] found no appreciable amounts of α-methylbenzyl alcohol present among the reaction products.

6. The product (or carbanilate) is oxidized with alkaline permanganate to benzoic-C^{14} acid, 70–80% yield, possessing 20.5-21.0% of the specific activity of the starting material. The rearrangement was 20.5–22.5% in four duplicate experiments with four different acid media: 10% hydrochlo-

ric acid, 10% perchloric acid, 50% acetic acid and glacial acetic acid. Roberts[3] has reported 27% rearrangement upon treating the hydrochloride in aqueous solution, or the free base in acetic acid solution, with nitrous acid (see ethanol-$C^{14}_{1/2}$). There is little or no hydrogen migration, in contrast with the reaction of propylamine.

C. Other Preparations

In a similar manner from *p*-nitrophenethylamine-α-C^{14} and *p*-methoxyphenethylamine-α-C^{14} are prepared[3] *p*-nitrophenethyl-α,β-$C^{14}_{1/2}$ alcohol and *p*-methoxyphenethyl-α,β-$C^{14}_{1/2}$ alcohol, with 5% and 33–45% rearrangement, respectively. Phenethylamine-α-C^{14} is nitrated[3] and isolated as the hydrochloride; yield 39%:, m.p. 212–214°. (*p*-Methoxyphenyl)acetonitrile-1-C^{14} (14 g.) is reduced to the amine with 9 g. of lithium aluminum hydride (see propylamine-1-C^{14} perchlorate); yield 5 g. (35%), b.p. 78–82° (0.7 mm.). An additional 1.5 g. of the amine is obtained as the hydrochloride; m.p. 213–214.5° from absolute alcohol-ether. Chromic acid oxidation is necessary for the degradation of the *p*-nitro-compounds.

Phenethylamine-α-C^{14} (b.p. 72–75° at 8 mm.) and hydrochloride (m.p. 219–219.6°) have been prepared by Roberts[3] in 33% yield, by reducing phenylacetonitrile-1-C^{14} with sodium and alcohol.

[1]L. F. Fieser, *Experiments in Organic Chemistry*, D. C. Heath and Co., New York, 1941, p. 381.

[2]A. Uffer and E. Schlittler, Helv. Chim. Acta, *31*, 1397 (1948).

[3]J. D. Roberts and C. M. Regan, J. Am. Chem. Soc., *75*, 2069 (1953).

α-BENZYL-α-$C^{14}_{1/1}$-BENZHYDROL-α-$C^{14}_{1/1}$ (1,1,2-Triphenylethanol-$C^{14}_{1/2}$)

$$C_6H_5C^*{\equiv}CC_6H_5 \xrightarrow{H_2O} C_6H_5C^*H_2C^*OC_6H_5 \xrightarrow{C_6H_5MgBr}$$

(a)

$$C_6H_5C^*H_2C^*(OH)(C_6H_5)_2$$

(b)

J. G. Burr, Jr., J. Am. Chem. Soc., *75*, 1990 (1953).

A. Procedure

(a) *2-Phenylacetophenone-1,2-*$C^{14}_{1/2}$, ($C^{14}_{1/2}$-*Deoxybenzoin*). A mixture of 0.50 g. of diphenylacetylene-C^{14} 5 ml. of concentrated sulfuric acid and 15 ml. of acetic acid is warmed until a clear solution results; then a few

crystals of mercuric chloride are added. After warming a few minutes more, the mixture is poured into water, and the product is filtered and air-dried; yield 0.500 g., m.p. 51–52° (Note 1).

(b) *α-Benzyl-α-$C^{14}_1/_1$-benzhydrol-α-$C^{14}_1/_1$*, *(1,1,2-Triphenylethanol-$C^{14}_1/_2$)*. The 2-phenylacetophenone-1,2-$C^{14}_1/_2$ is added to a Grignard solution, prepared from 3 g. of bromobenzene and 0.5 g. of magnesium. The mixture is stirred overnight, then hydrolyzed, and the product is collected and recrystallized from hexane; yield 0.480 g. of needles, m.p. 88°. Degradation studies indicate that the two positions are uniformly labeled (Note 2).

B. Notes

1. A mixture melting with an authentic sample shows no depression, whereas the mixture melting point with diphenylacetylene (m.p. 56–57°) is depressed.

2. Directions are given by Burr for oxidation of the carbinol with chromic acid to form benzophenone-C^{14} (oxime, m.p. 141°) and benzoic-C^{14} acid (benzoyl-C^{14} chloride and *N*-phenylbenzamide-C^{14}, m.p. 161°). Any isotope effect in the addition of water to diphenylacetylene-C^{14} is less than 1–2%.

1,2,2-TRIPHENYLETHANOL-$C^{14}_1/_2$

$$(C_6H_5)_2CHCl \xrightarrow{Cu(C^*N)_2} (C_6H_5)_2CHC^*N \xrightarrow{H_2SO_4} (C_6H_5)_2CHC^*OOH \xrightarrow{SOCl_2}$$
(a)

$$(C_6H_5)_2CHC^*OCl \xrightarrow{(C_6H_5)_2Cd} (C_6H_5)_2CHC^*OC_6H_5 \xrightarrow{LiAlH_4}$$
(b) (c)

$$(C_6H_5)_2CHC^*HC_6H_5 \xrightarrow[NH_3]{Na} (C_6H_5)_2CHC^*HC_6H_5 \xrightarrow{CH_3\text{-}C_6H_4\text{-}SO_2Cl}$$
(d) (OH / ONa)

$$(C_6H_5)_2CHC^*HC_6H_5 \xrightarrow{HOAc} (C_6H_5)_2C^*HC^*HC_6H_5 \xrightarrow{LiAlH_4}$$
(e) (OSO$_2$-C$_6$H$_4$-CH$_3$) (f) (OAc)

$$(C_6H_5)_2C^*HC^*HC_6H_5 \xrightarrow{KMnO_4} (C_6H_5)_2C^*O + C_6H_5C^*OOH$$
(g) (OH)

W. A. Bonner and C. J. Collins, J. Am. Chem. Soc., 75, 5372 (1953).

A. Procedure (Note 1)

(a) *Diphenylacetic-1-C^{14} Acid.* A mixture of 54 g. (266 mmoles) of freshly distilled chlorodiphenylmethane (Note 2) and 20.6 g. (239 mmoles) of cuprous cyanide-C^{14} is heated for 2 hours with a bath preheated to 200–210°. The mixture is diluted with acetone, and the filtered solution is evaporated.

The residual crude diphenylacetonitrile-1-C^{14} (Note 3) is stirred under reflux for 3.5 hours with 250 ml. of 50% (by vol.) sulfuric acid. The mixture is continuously extracted with ether, and the extract is concentrated and stirred with warm saturated sodium bicarbonate solution. The aqueous solution is stored for several days, filtered, and acidified to precipitate the product, which is collected, washed with water and dried; yield 25.2 g. (50%), m.p. 145–146.5°.

(b) *Diphenylacetyl-1-C^{14} Chloride.* To a solution of 50 g. of diphenylacetic-1-C^{14} acid in 150 ml. of dry benzene is added gradually 80 ml. of thionyl chloride. The mixture is refluxed for 7 hours and evaporated under reduced pressure (100°), the last traces of reagent being removed by codistillation with 125 ml. of benzene. The residue is crystallized from 150 ml. of hexane (Norit) by seeding and storing at 0°; yield 42.1 g. (78%), m.p. 56–56.6°.

(c) *2,2-Diphenylacetophenone-1-C^{14}* (Note 4). To an ice-cooled, stirred benzene solution of diphenylcadmium, prepared from 201 mmoles of anhydrous cadmium chloride (Note 5), is added 42.1 g. of diphenylacetyl-1-C^{14} chloride in 90 ml. of benzene during 10 minutes. The mixture is stirred for 1 hour at room temperature, refluxed for 30 minutes, cooled at 0° and hydrolyzed by the slow addition of water and sufficient hydrochloric acid to dissolve the magnesium salts. The benzene extract is washed successively with water, 5% sodium hydroxide and water, then dried over sodium sulfate, decolorized with Norit and concentrated by flash distillation. A solution of the residue (57.6 g.) in 58 ml. of hot alcohol and stored at 0° for 2 days; yield 39.9 g. (79%), m.p. 136–136.5°.

(d) *1,2,2-Triphenylethanol-1-C^{14}*. To a stirred mixture of 7 g. of lithium aluminum hydride, 235 ml. of absolute ether, and glass beads is added dropwise a solution of 35 g. of 2,2-diphenylacetophenone-1-C^{14} in 235 ml. of warm benzene during 20 minutes. The mixture is stirred for 45 minutes and decomposed by the gradual addition of ethyl acetate, water and hydrochloric acid. The organic layer is separated, washed, dried and concentrated. The clear oil (103%) is crystallized from 155 ml. of acetic acid; yield 35.1 g., m.p. 79–84° (Note 6). The product is recrystallized from acetic acid and dried under vacuum over sulfuric acid (Note 7). Degradation studies show that there is no isotopic rearrangement (Note 8).

(e) *1,2,2-Triphenylethyl-1-C^{14} p-Toluenesulfonate.* To a stirred, cooled solution of about 0.1 g. of sodium in 85 ml. of liquid ammonia is added dropwise a portion of a solution of 5.00 g. of 1,2,2-triphenylethanol-1-C^{14} in 100 ml. of absolute ether until the blue color is discharged (Note 9). An additional small quantity of powdered sodium is added, and the process is repeated until all the carbinol has been added. The color due to sodium is discharged with a small amount of the solid carbinol; then 50 ml. of ether is added, and the ammonia is evaporated slowly. The ether suspension is stirred in an air stream to remove residual ammonia, then is treated dropwise with a solution of 4.00 g. of distilled *p*-toluenesulfonyl chloride in 35 ml. of absolute ether. The mixture is stirred for 30 minutes and filtered through Celite, the cake being washed three times by resuspension in boiling ether. The filtrate is concentrated to 50 ml. and stored to crystallize the product; yield 2.57 g., m.p. 74–75°. A second crop, 0.20 g., m.p. 70–71°, is obtained by concentrating the mother liquor to 10 ml. and diluting with 30 ml. of ligroin. The combined crude product is purified by dissolution in 8 ml. of acetone and dilution with 30 ml. of ligroin; yield 2.45 g., m.p. 78–79° (Note 10). The molar specific activity is the same as that of the starting material. Degradation studies indicate that at least 91%, and probably all of the activity, is located at C-1 (Note 11).

(f) *1,2,2-Triphenylethyl-$C^{14}_{1/2}$ Acetate.* A mixture of 0.90 g. (2.10 mmoles) of 1,2,2-triphenylethyl-1-C^{14} *p*-toluenesulfonate and 8 ml. of glacial acetic acid containing 0.1 ml. of acetic anhydride is heated on a steam-bath for 1 hour. The mixture is diluted with water to the point of turbidity and set aside to crystallize; yield 0.43 g. (59%), m.p. 142–145°. Recrystallization gives 0.39 g., m.p. 154–155° (Note 12).

(g) 1,2,2-*Triphenylethanol-$C^{14}_{1/2}$.* To a stirred slurry of excess lithium aluminum hydride in ether is added 0.66 g. of 1,2,2-triphenylethyl-$C^{14}_{1/2}$ acetate in portions. The mixture is stirred for 5 minutes and decomposed by the cautious addition of acetone, water and hydrochloric acid. Evaporation of the washed ether layer gives a clear syrup which is crystallized from acetic acid; yield 93%, m.p. 87–88.5°. Degradation studies indicate that 48% of the activity is located at C-2.

B. Notes

1. The preparation are incidental to a study of the mechanism of intramolecular rearrangement during dehydration and solvolysis in the triphenylethanol series; also see 1,2-bis(phenyl-$C^{14}_{1/4}$)-2-phenylethyl acetate.

2. The reagent is prepared by reacting benzhydrol with thionyl chloride in benzene.

3. Diphenylacetonitrile, m.p. 76°, b.p. 181° (12 mm.).

4. The procedure is adapted from the method of Cason.[1]

5. To an ice-cooled Grignard solution containing 402 mmoles of phenylmagnesium bromide is added slowly 36.9 g. of anhydrous cadmium chloride. Glass beads are added, and the mixture is stirred for 10 minutes at 0° and 15 minutes at room temperature. The bulk of the ether is distilled, 275 ml. of anhydrous benzene is added, and distillation is continued until the vapor reaches 68°; then 50 ml. of benzene is added.

6. Slight dilution of the mother liquor yields a second crop (3.8 g.) containing some 1,1,2,2-tetraphenylethanol (m.p. 235.5°), which arises from the action of phenylmagnesium bromide on diphenylacetyl chloride.

7. One molecule of acetic acid is lost, and the melting point drops from 87.5–88.5° to 82.5–83.5°. Recrystallization from acetic acid again gives the higher melting point, and the molar specific activities correspond to carbinol solvation. Under dehydrating conditions the nondesiccated alcohol undergoes acetylation with its own solvent of crystallization, forming 1,2,2-triphenylethyl-1-C^{14} acetate, m.p. 156.5–157°. Directions are given for acetylating with acetic anhydride in pyridine[2,3] or with acetic acid and anhydrous sodium acetate[3] (see 1-phenyl-$C^{14}_{1/4}$-2,2-diphenylethyl acetate); yield 96–99%. Deacetylation by hydrolysis with alcoholic sodium hydroxide, or by treatment with excess ethereal lithium aluminum hydride, gives quantitative yields of the alcohol; oxidative degradation indicates that there is no isotopic rearrangement.

8. Directions are given by Bonner for oxidizing the compound with permanganate in dilute acetone solution, forming benzophenone (103%) and benzoic-C^{14} acid (99%); also see triphenylethylene-$C^{14}_{1/2}$, Note 6. The molar specific activity of the acid is 92% of that of the starting material, as a consequence of partial oxidation of the benzophenone. This fact was ascertained by subjecting benzophenone-C^{14} to the conditions of oxidation and working up the mixture with carrier benzoic acid.

9. The apparatus consists of a three-necked flask containing 25 g. of glass beads and equipped with a Hershberg stirrer, reflux condenser, calcium chloride guard tube and dropping funnel.

10. The highest melting point noted for such preparations is 82.5–83°. The compound appears to deteriorate upon standing for more than a few hours.

11. Directions are given by Bonner for reducing the tosylate with excess lithium aluminum hydride in ether to 1,1,2-triphenylethane-$C^{14}_{1/2}$, m.p. 51–52°. The ethane derivative is dehydrogenated by heating with an equal weight of chloranil in xylene for 3 days, and the triphenylethylene-$C^{14}_{1/2}$ then is degraded by permanganate oxidation. The molar specific activity of the benzophenone-C^{14} 2,4-dinitrophenylhydrazone is 9% of that of the tosylate. Since Cram[4] has reported that phenyl migration may accompany tosylate reduction to the hydrocarbon stage by the use of lithium aluminum hydride, it is not unlikely that all the activity is located at C-1 in the tosylate.

12. The effect appears to be catalytic due to the *p*-toluenesulfonic acid formed during solvolysis of the tosylate. The same rearranged compound is obtained (93% yield) by heating an equimolar mixture of *p*-toluenesulfonic acid hydrate and 1,2,2-triphenylethyl-1-C^{14} acetate in acetic acid for 1 hour on a steam-bath. There is no tendency for isotopic rearrangement under solvolytic conditions when one or more equivalents of sodium acetate are present per molecule of the acetate. The acetolysis reaction is quite rapid, for it has been observed during recrystallization of the tosylate from acetic acid on one occasion.

C. Other Preparations

In a similar manner from bromobenzene-$C^{14}_{1/4}$ are prepared[5] the ring-labeled analogues: bis(phenyl-$C^{14}_{1/4}$)cadmium, 2,2-diphenylacetophenone-1′,2′,3′,4′-$C^{14}_{1/4}$ (80%, m.p. 136–137°; results are unsatisfactory unless the labeled diphenylcadmium is in about 30% excess), 1-phenyl-$C^{14}_{1/4}$-2,2-diphenylethanol (90%) and 1-phenyl-$C^{14}_{1/4}$-2,2-diphenylethyl *p*-toluenesulfonate (m.p. 79–80°), all without isotopic rearrangement. Hydrolysis of the tosylate by refluxing in aqueous-acetone for 2.5 hours gives a quantitative yield of 1,2-bis(phenyl-$C^{14}_{1/4}$)-2-phenylethanol, m.p. 87–88° (23.6% activity at 2-phenyl). Acetolysis of the tosylate in acetic acid in the presence of a three-fold excess of anhydrous sodium acetate gives 1,2-bis(phenyl-$C^{14}_{1/4}$)-2-phenylethyl acetate (yield 82%, m.p. 155–156°). Degradation of the acetate gives 1,2-bis(phenyl-$C^{14}_{1/4}$)-2-phenylethanol (yield 87%, m.p. 87–88°, 47.1% activity at 2-phenyl).

1,2,2-Triphenylethanol-$C^{14}_{1/2}$ (22.2% activity at C-2) has been prepared by refluxing an aqueous acetone solution of 1,2,2-triphenylethyl-1-C^{14} *p*-toluenesulfonate.

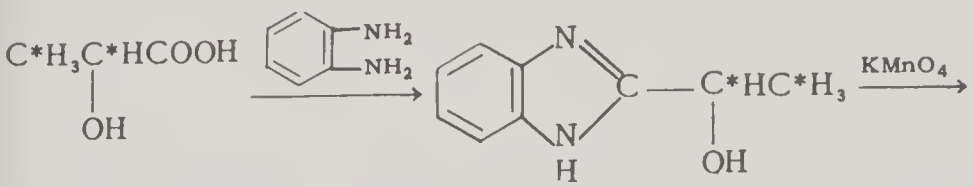
[1,1,2-]Triphenylethylene-$C^{14}_{1/2}$ (49.3% activity at C-1) has been prepared by heating 1,2,2-triphenylethyl-1-C^{14} *p*-toluenesulfonate with 98% formic acid.

The preparation of 2,2-diphenylacetophenone-1-C^{14} by the method of Lane[6] has been described by Collins.[7] 1,1,2-Triphenylethanol-1-C^{14} was brominated with *N*-bromosuccinimide, and the 2-bromo-1,1,2-triphenylethanol-1-C^{14} (0.714 g.) was subjected to semipinacolic dehydrobromination by storage with 0.326 g. of mercuric nitrate in 85 ml. of 70% aqueous dioxane for 6 days at room temperature; yield 0.273 g., m.p. 136–137.5°. Degradation by permanganate oxidation (4 days at room temperature) gave benzoic-C^{14} acid having the same molar specific activity.

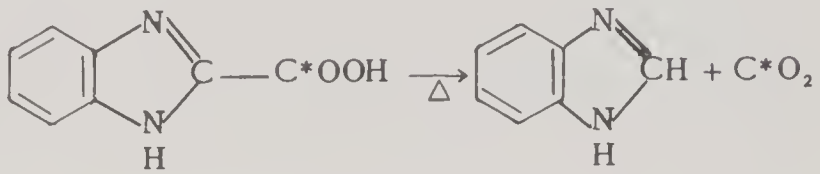
[1]J. Cason, J. Am. Chem. Soc., *68*, 2080 (1946); Chem. Revs., *40*, 15 (1947).
[2]W. A. Bonner and C. J. Collins, J. Am. Chem. Soc., *77*, 99 (1955).
[3]W. A. Bonner and C. J. Collins, J. Am. Chem. Soc., *75*, 5372 (1953).
[4]D. J. Cram, J. Am. Chem. Soc., *74*, 2149, 2152 (1952).
[5]C. J. Collins and W. A. Bonner, J. Am. Chem. Soc., *77*, 92 (1955).

[6]J. F. Lane and D. R. Walters, J. Am. Chem. Soc., 73, 4234 (1951).
[7]C. J. Collins and W. A. Bonner, J. Am. Chem. Soc., 75, 5379 (1953).

α-METHYL-$C^{14}_{1/1}$-2-BENZIMIDAZOLEMETHANOL-α-$C^{14}_{1/1}$
[2-(1-Hydroxyethyl-$C^{14}_{1/2}$)benzimidazole]



(a)



(b)

S. Roseman, J. Am. Chem. Soc., 75, 3854 (1953).

A. Procedure (Note 1)

(a) *α-Methyl-$C^{14}_{1/1}$-2-benzimidazolemethanol-α-$C^{14}_{1/1}$.* Lactic-2,3-$C^{14}_{1/2}$ acid is condensed with excess *o*-phenylenediamine dihydrochloride by a procedure that is identical with that described for acetic acid (see benzimidazole-2-C^{14}), except that the period of heating in a sealed tube is omitted. As an alternative to isolation of the silver salt, the product may be isolated by ether extraction from the neutralized mixture; yield 0.40 g. (m.p. 179–180°) from 0.40 g. of lactic acid. Purification may be achieved by vacuum sublimation, or by recrystallization from butyl ether, giving a product melting at 180–181°. Degradation by hypoiodite isolates C-3 of lactic acid (Note 2).

(b) *2-Benzimidazolecarboxylic-C^{14} Acid.* To a boiling solution of 0.810 g. of the carbinol and 0.20 g. of sodium carbonate in 50 ml. of water is added, at one time, a solution of 2.90 g. of potassium permanganate in 100 ml. of water. The mixture is boiled for 3 minutes and then heated on a steam-bath for 30 minutes. The excess permanganate is destroyed with a few drops of ethanol, and the solution is decolorized with a small amount of carbon. The filtrate is adjusted to pH 6 with acetic acid and placed in a refrigerator. After 2 days the product is collected; yield 0.675 g., m.p. 174°(dec.) (Note 3).

B. Notes

1. The original paper describes the preparation of singly labeled products derived from lactic-2-C^{14} acid and lactic-3-C^{14} acid, in developing this method for the degradation and characterization of micro amounts of impure labeled lactic acid. The mixture, lactic-2,3-$C^{14}_{1/2}$ acid, is used for convenience in illustrating the method.

2. α-Methyl-$C^{14}_{1/1}$-2-benzimidazolemethanol-α-$C^{14}_{1/1}$ (0.054 g.) is treated, in alkaline dioxane solution at 60°, for 30 minutes with an excess of iodine-potassium iodide solution. The excess iodine is then destroyed with a few drops of 10% sodium hydroxide solution, and the mixture is diluted with water and set aside for 15 minutes. The yield of iodoform-C^{14}, originating as the C-3 of lactic acid-2,3-$C^{14}_{1/2}$, is 0.022 g., m.p. 117–119°.

3. The yield is 0.025 g. on the 0.041-g. scale. This compound loses two molecules of water of crystallization at 80–90°, and prolonged heating at this temperature slowly decarboxylates the acid (C-2 of lactic acid). For the degradation of this acid see benzimidazole-2-C^{14}.

1,2,2-TRIPHENYL-1,2-ETHANEDIOL-1-C^{14}

$$(C_6H_5)_2CHC^*OC_6H_5 \xrightarrow[HOAc]{HNO_3} (C_6H_5)_2\overset{OH}{\overset{|}{C}}C^*OC_6H_5 \xrightarrow{LiAlH_4} (C_6H_5)_2\overset{OH}{\overset{|}{C}}-\overset{OH}{\overset{|}{C^*}}HC_6H_5$$

(a) (b)

C. J. Collins, J. Am. Chem. Soc., 77, 5517 (1955).

A. Procedure

(a) *2-Hydroxy-2,2-diphenylacetophenone-1-C^{14}.* 2,2-Diphenylacetophenone-1-C^{14} (5.00 g.) is oxidized by a suitable modification of the method of Biltz,[1] which follows.

To a solution of 6 g. of 2,2-diphenylacetophenone in 25 g. of glacial acetic acid is added 9 g. of concentrated nitric acid. The mixture is boiled gently for 15 minutes and poured into water. The oily product is collected and crystallized from alcohol, m.p. 84–85°.

(b) *1,2,2-Triphenyl-1,2-ethanediol-1-C^{14}.* The crude ketol is reduced with lithium aluminum hydride in ether; yield 4.7 g. (88% based on 2,2-diphenylacetophenone), m.p. 167–168° from alcohol-water.

[1]H. Biltz, Ber., *32*, 650 (1899).

1,2-PROPANEDIOL-1-C^{14}
(Propylene-1-C^{14} Glycol)

$$\underset{\displaystyle OH}{CH_3\overset{|}{C}HC^*OOH} \xrightarrow{LiAlH_4} \underset{\displaystyle OH}{CH_3\overset{|}{C}HC^*H_2OH}$$

H. Rudney, J. Biol. Chem., *210*, 353 (1954)

A. Procedure (Note 1)

An 80% aqueous solution containing 20 mmoles of lactic-1-C^{14} acid is mixed thoroughly with 40 g. of anhydrous magnesium sulfate, and the solid is packed in a Soxhlet extraction apparatus over 10 g. of magnesium sulfate, making a column 140 × 26 mm. The lactic acid is extracted during 7 hours by boiling an absolute ether solution containing 30 mmoles of lithium aluminum hydride. The reaction mixture is hydrolyzed with an excess of water, and the suspension is adjusted to pH 7 with 10 *N* sulfuric acid, then mixed thoroughly with Celite No. 535 (2 g. per ml.) and extracted with ether for 14 hours. The product is collected by concentrating the extract and fractionally distilling, b.p. 80–84° (9–13 mm.). Purity is established by paper chromatography, radioautography, and analysis by periodic acid oxidation (Note 2); there is no isotopic rearrangement.

B. Notes

1. The reduction of lactic acid with sodium and alcohol gives poor yields.

2. The amount of periodate[1] taken up, and the acetaldehyde and formaldehyde-C^{14} formed are equivalent. Acetaldehyde is determined by titration of the bisulfite complex, and formaldehyde is determined by steam distillation and oxidation to formate with sodium hypoiodate.

The ascending paper chromatographic method utilizes ether saturated with water,[2] while a more satisfactory method employs a Celite column.[3]

C. Other Preparations

The preparation of 1-propanol-1-C^{14} and 1-propanol-2-C^{14} by reduction of the appropriate propionic acids has been reported by Kummer.[4]

[1] *Organic Reactions*, Vol. II, Wiley, New York, 1944, Chapt. 8.
[2] L. Hough, Nature, *165*, 400 (1950).
[3] A. C. Neish, Can. J. Res., *28B*, 535 (1950).
[4] J. T. Kummer and P. H. Emmett, J. Am. Chem. Soc., *75*, 5177 (1953).

2-METHYL-1,2-PROPANEDIOL-2-C^{14}

$$CH_3C^*OCl \xrightarrow{CH_3MgCl} (CH_3)_3C^*OH \xrightarrow{HCl} (CH_3)_3C^*Cl \xrightarrow{NaOC_2H_5}$$
(a) (b)

$$CH_2{=}C^*(CH_3)_2 \xrightarrow[KOH]{HCO_3H} CH_2C^*(CH_3)_2$$
OH OH
(c)

J. D. Roberts, R. E. McMahon and J. S. Hine, J. Am. Chem. Soc., 72, 4237 (1950).

A. Procedure

(a) *2-Methyl-2-propanol-2-C^{14}* (Note 1). Acetyl-1-C^{14} chloride (52.0 g., 0.66 mole) is added to a stirred, ice-cooled solution of 1.60 moles of methylmagnesium chloride in 1 l. of dry ether under nitrogen. The product is isolated according to the procedure described for 2-methyl-2-butanol-2-C^{14}; yield 29.0 g. (59%), b.p. 78–82.5°.

(b) *2-Chloro-2-methylpropane-2-C^{14}*. 2-Methyl-2-propanol-2-C^{14} is converted to the C_1^{14}-*t*-butyl chloride with hydrochloric acid by the procedure of Norris and Olmstead.[1] The yield is 73%, b.p. 50–50.5°, n_D^{25} 1.3818.

(c) *2-Methyl-1,2-propanediol-2-C^{14}* (Note 2). The glycol is formed without isolation of the volatile intermediate 2-methylpropene-2-C^{14}. A mixture of 6.6 g. of 2-chloro-2-methylpropane-2-C^{14} and a solution of sodium ethoxide, prepared from 2.3 g. of sodium and 50 ml. of ethanol, is heated in a flask fitted with a reflux condenser and nitrogen inlet tube. The top of the condenser is connected, through an ice-cooled trap, to the inlet of a second flask containing 7.5 ml. of 35% hydrogen peroxide and 30 ml. of 87% formic acid. This flask is equipped with a magnetic stirrer and an ice water-cooled reflux condenser surmounted by a Dry Ice-cooled reflux condenser. The dehydrohalogenation reaction is carried out for 10 hours; then the system is flushed with nitrogen to transport any remaining 2-methylpropene-2-C^{14} into the hydroxylation flask. This second reaction mixture is allowed to stand for several hours, before being worked up in the manner described for 2-methyl-2,3-butanediol-2-C^{14}; yield 2.5 g. (39%), b.p. 96–98° (30 mm.). Degradation studies indicate that there is no carbon rearrangement (Note 3).

B. Notes

1. See the similar preparation of 2-methyl-2-butanol-2-C^{14}.
2. See the similar preparation of 2-methyl-2,3-butanediol-2-C^{14}.
3. The degradative procedure parallels that described for 2-methyl-2, 3-butanediol-2-C^{14}; see Note 8.

C. Other Preparations

In a similar manner, without rearrangement, are prepared 2-chloro-2-methylbutane-1-C^{14} and 2-chloro-2-methylbutane-2-C^{14}; yield 85–90%, b.p. 41–43° (180 mm.), n_D^{25} 1.4012.

[1]*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 144.

1,2,3-PROPANETRIOL-1-C^{14} (Glycerol-1-C^{14})

METHOD I

$$BrCH_2C^{\circ}OONa \xrightarrow{NaC^*N} NC^*CH_2C^{\circ}OOH \xrightarrow[2.\ CaCl_2]{1.\ NaOH}$$

$$\left[\begin{array}{c} C^*OO \\ \diagup \\ CH_2 \\ \diagdown \\ C^{\circ}OO \end{array}\right] = Ca^{++} \xrightarrow[HCl]{C_2H_5OH} \begin{array}{c} C^*OOC_2H_5 \\ | \\ CH_2 \\ | \\ C^{\circ}OOC_2H_5 \end{array} \xrightarrow{Pb(OAc)_4}$$

$$\begin{array}{c} C^*OOC_2H_5 \\ | \\ CHOCOCH_3 \\ | \\ C^{\circ}OOC_2H_5 \\ \text{(a)} \end{array} \xrightarrow{LiAlH_4} \begin{array}{c} C^*H_2OH \\ | \\ CHOH \\ | \\ C^{\circ}H_2OH \\ \text{(b)} \end{array}$$

L. I. Gidez and M. L. Karnovsky, J. Am. Chem. Soc., 74, 2413 (1952).

A. Procedure (Note 1)

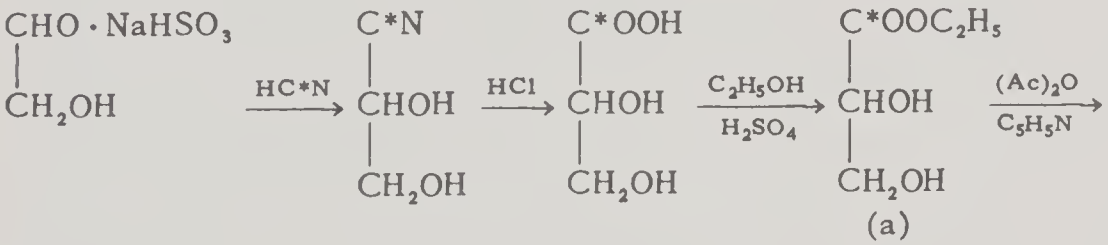
(a) *Ethyl Acetoxymalonate-1-C^{14}*, (*Ethyl Acetyltartronate-1-C^{14}*). The oxidation of ethyl malonate-1-C^{14} is carried out, in 83% yield, according to the directions of Dimroth and Schweizer,[1] which follow.

Ethyl malonate (16 g.) is readily oxidized at water-bath temperature with 44 g. of lead tetraacetate and 40 ml. of glacial acetic acid. The mixture is cooled and diluted with water, and the product is extracted into ether, which then is washed with dilute alkali, dried, concentrated and vacuum-distilled; b.p. 134–136° (17 mm.).

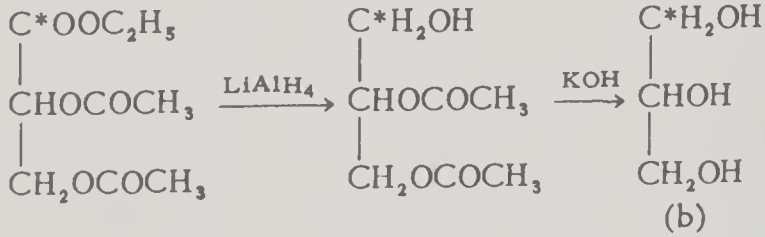
(b) *1,2,3-Propanetriol-1-C*^{14}, (*Glycerol-1-C*^{14}). To a stirred, ice-cooled solution of 7 g. (0.2 mole) of lithium aluminum hydride[2] in 250 ml. of absolute ether (Note 2) is added 17.9 g. (82 mmoles) of ethyl acetoxymalonate-1-C^{14} in 200 ml. of absolute ether over a period of 1 hour. The mixture is refluxed for 1 hour, then cooled with an ice-bath and decomposed by the slow addition of 200 ml. of water. The aqueous layer is separated (Note 3), adjusted to pH 7 with sulfuric acid, filtered and passed through an anion and cation exchange column to remove all salts (Note 4). The ion-free aqueous solution of product is lyophilized (Note 5), the final 20–30 ml. of water being removed under vacuum over phosphorus pentoxide. The product is 7.4 g. of pale yellow liquid, assaying 86.6% purity (note 6). The yield is 6.4 g. (85%), 40% based on cyanide-C^{14}, or 30% based on barium carbonate-C^{14}. Filter paper partition chromatography, by the procedure of Hough,[3] indicates the presence of two trace contaminants, believed to be derived from the column.

1,2,3-Propanetriol-2-C^{14} (Note 7) is prepared from bromoacetic-2-C^{14} acid by the same procedure.

METHOD II



(a)



(b)

A. P. Doerschuk, J. Am. Chem. Soc., 73, 821 (1951).

A. Procedure

(a) *Ethyl Glycerate-1-C*^{14} (Note 8). To 10 ml. of an aqueous solution of 5.0 mmoles of sodium hydroxide and 2.5 mmoles of sodium cyanide-C^{14} is

added 5.0 ml. of 16.7% aqueous sodium bisulfite solution of glycolaldehyde (3.75 mmoles); then the mixture is stored at 35° for 26 hours (Note 9). The mixture is acidified with hydrochloric acid and quickly evaporated to dryness at 45° under vacuum. The glyceronitrile-1-C^{14}, (glycolaldehyde cyanohydrin-C^{14}), is hydrolyzed under nitrogen by heating with 10 ml. of 6 *N* hydrochloric acid for 4 minutes in a boiling water-bath; then the solution is quickly evaporated to dryness at 45° under vacuum.

The dry glyceric-1-C^{14} acid is refluxed for 12 hours with a mixture of 25 ml. of 2.5 *N* sulfuric acid in absolute ethanol and 5 ml. of toluene, the water formed being removed azeotropically. The acid is neutralized with excess barium carbonate, and the solution is concentrated to 5 ml. under vacuum at 25°. Absolute ether (45 ml.) is added with stirring, and the supernatant solution is separated after centrifuging. The precipitate is washed three times by treatment with 5 ml. of absolute ethanol and precipitation with ether. The combined supernatant liquid is concentrated at 25° under reduced pressure, then distilled at 4 mm. with 0.30 g. of carrier ethyl glycerate from a small amount of barium carbonate (Note 10). The distillate is mixed with 10 ml. of absolute ether and centrifuged, and the clear solution is concentrated and distilled (4 mm.); radiochemical yield 19%, 0.33 g. (Note 11).

(b) *1,2,3-Propanetriol-1-*C^{14}. A mixture of 0.090 g. of ethyl glycerate-1-C^{14}, 1 g. of acetic anhydride and 0.6 g. of pyridine is refluxed for 3 hours. Carrier ethyl 0,0′-diacetylglycerate (0.30 g.) is added, and the mixture is evaporated to dryness under vacuum.

The residual ethyl 2,3-diacetoxypropionate-1-C^{14}, (ethyl 0,0′-diacetylglycerate-1-C^{14}), is twice distilled over silver carbonate (4 mm.), dissolved in 2 ml. of absolute ether and added dropwise to a solution of 0.20 g. of lithium aluminum hydride in 40 ml. of absolute ether in a 125-ml. flask equipped with a magnetic stirrer, reflux condenser and dropping funnel. The mixture is stirred for 30 minutes, then hydrolyzed by the cautious addition of 2 ml. of water, followed by 5.2 ml. of cold 25% sulfuric acid. All but about 5 ml. of ether is evaporated in a stream of nitrogen. A cold 10% methanolic solution of potassium hydroxide (13.2 ml.) is added slowly, and the white precipitate is removed by centrifuging and washed twice with isopropyl alcohol. The combined solution is made faintly acid with 25% sulfuric acid, and 3 g. of barium carbonate is added, followed by the gradual addition of 10 g. of anhydrous magnesium sulfate, with cooling and stirring. After standing for 1 hour, the solids are removed by centrifuging and washed with isopropyl alcohol. The combined solution is evaporated to dryness under vacuum at 25°, and the residue is dissolved in 7 ml. of isopropyl alcohol. The solution is made alkaline to litmus with 10% methanolic potassium hydroxide, centrifuged and refluxed for 30 minutes on a water-bath. The mixture is faintly acidified

with 25% sulfuric acid, and an excess of barium carbonate is added. The solids are separated by centrifuging, and the solution is evaporated to dryness at 25° under vacuum. The residue is dissolved in 5 ml. of water containing 0.20 g. of carrier glycerol, and the solution is washed three times (each) with chloroform, ligroin (b.p. 70–90°) and ether. The aqueous solution is made slightly alkaline with potassium carbonate and concentrated, and the residue is distilled (4 mm.). The distillate is dissolved in methanol, decolorized with Norit A (15 minutes) and dried by azeotropic distillation of a few ml. of benzene, and the product is distilled (4 mm.); yield 0.27 g., 63.4% radiochemical yield (Note 12).

METHOD III

$$HC^*HO \xrightarrow[KOH]{CH_3NO_2} (HOC^*H_2)_3CNO_2 \xrightarrow{NaOCH_3} \underset{(a)}{[(HOC^*H_2)_2CNO_2]^-}\ Na^+ \xrightarrow[Ni]{H_2}$$

$$(HOC^*H_2)_2CHNH_2 \cdot HOAc \xrightarrow{(COOH)_2} (HOC^*H)_2CHNH_2 \cdot \tfrac{1}{2}(COOH)_2 \xrightarrow[HCl]{Ba^{++}}$$

$$\underset{(b)}{(HOC^*H_2)_2CHNH_2 \cdot HCl} \xrightarrow{CH_3(CH_2)_4COCl}$$

$$\underset{(c)}{[CH_3(CH_2)_4COOC^*H_2]_2CHNH_2 \cdot HCl} \xrightarrow{HONO}$$

$$\underset{(d)}{[CH_3(CH_2)_4COOC^*H_2]_2CHOH} \xrightarrow[IRA\text{-}400]{CH_3OH} \underset{(e)}{(HOC^*H_2)_2CHOH}$$

H. Schlenk and B. W. De Haas, J. Am. Chem. Soc., 73, 3921 (1951).

A. Procedure

(a) *2-Nitro-2-sodium-1,3-propanediol-1,3-C_2^{14}* (Note 13). To a stirred mixture of 13.47 g. (0.449 mole) of crude paraformaldehyde-C^{14} and 8.54 g. (0.14 mole) of nitromethane in 110 ml. of anhydrous methanol is added 8–10 drops of 50% potassium hydroxide. The mixture is heated for 45 minutes on a steam-bath, then decanted from the unreacted insoluble particles (Note 14), cooled to −5°, and treated with a solution of 4.02 g. of sodium in 65 ml. of absolute methanol with stirring over a period of 20 minutes. After standing for 6 hours at −5°, the product is filtered off; yield 27 g., 58% (Note 15). The mother liquor is neutralized with 7.8 *N* hydrogen chloride in methanol, then mixed with the unreacted paraformal-

dehyde-C^{14}, and the preparation is repeated by adding 3.05 g. (0.05 mole) of nitromethane. The yield is 8 g. and another recycling gives 1 g., making the combined total yield 36 g. (77%).

(b) *2-Amino-1,3-propanediol-1,3-C_2^{14} Hydrochloride.* To a mixture of 18 g. of 2-nitro-2-sodium-1,3-propanediol-1,3-C_2^{14} and 225 ml. of absolute methanol is added 2.1 equivalents of glacial acetic acid and 4 g. of Raney nickel. Hydrogenation is effected in about 6 hours at room temperature at 37–20 p.s.i. (Note 16). The amine oxalate is precipitated from the filtered solution by addition of a concentrated ethereal solution of anhydrous oxalic acid (10% excess) and storage overnight at $-20°$.

The 2-amino-1,3-propanediol-1,3-C_2^{14} oxalate (34 g. from two batches) is dissolved in 315 ml. of water and treated with sufficient barium chloride solution to react with one-half of the oxalate, followed by barium hydroxide solution until the pH is above 11.5. The barium oxalate is filtered off, and the remaining barium is removed as the carbonate. The filtrate is acidified with hydrochloric acid to pH 1.9, and the water is removed by lyophilization. The residue is dried over potassium hydroxide and phosphorus pentoxide, then extracted with a total of 120 ml. of hot absolute ethanol to remove sodium chloride. The product is crystallized at 5° and collected, and a second crop is obtained by slow addition of 55 ml. of absolute ether; yield 13.17 g. (59%), m.p. 93–97° (Note 17).

(c) *2-Amino-1,3-dihexanoöxypropane-1,3-C_2^{14} Hydrochloride.* The dried 2-amino-1,3-propanediol-1,3-C_2^{14} hydrochloride is treated with 1.95 molar equivalents of hexanoyl chloride at 90°. The mixture solidifies as the evolution of hydrogen chloride ceases, and a small amount of unreacted acid chloride is removed in a vacuum desiccator over potassium hydroxide. The product is recrystallized from anhydrous dioxane, after filtering off any trace of unreacted amine salt. The mother liquor is concentrated, diluted with ether and cooled to crystallize a second crop. Repetition of this procedure gives a total yield of 27.4 g. (81.8%), m.p. 113–118° (Note 18).

(d) *1,3-Dihexanoin-1,3-C_2^{14}.* To an ice-cooled solution of 84.7 mmoles of 2-amino-1,3-dihexanoöxypropane-1,3-C_2^{14} hydrochloride in 138 ml. of 50% acetic acid is added slowly a concentrated aqueous solution of 11.66 g. (169 mmoles) of sodium nitrite, the temperature being kept below 8°. When reaction is complete (10 minutes), the yellow, oily, upper layer is separated, and an additional 5.83 g. of sodium nitrite is added to the aqueous phase. After standing for 1.5 hours, the mixture is extracted with ether, and the small amounts of nitrous oxides are removed by washing with a 10% solution of sulfamic acid. The first oily layer is treated similarly in ether solution; then the combined extract is dried over sodium sulfate and evaporated under vacuum to remove ether and most of the hexanoic acid. The yield of crude product is 24.89 g.

(e) *1,2,3-Propanetriol-1,3-C_2^{14}*, (*Glycerol-1,3-C_2^{14}*). To a solution of the crude 1,3-dihexanoin-1,3-C_2^{14} in 300 ml. of methanol is added 60 g. of activated Amberlite IR-A-400 (Note 19). After 22 hours of shaking, the resin is filtered off and washed with 100 ml. of methanol (Note 20). The combined solution is evaporated under vacuum at room temperature to remove the solvent and most of the methyl hexanoate formed by interesterification. The remaining hexanoic acid, water and methyl hexanoate are removed in a vacuum desiccator over potassium hydroxide and phosphorus pentoxide. The yellow crude product (4.82 g.) is distilled (3 mm.) with a bath temperature of 140–155°; yield 3.95 g., 50.6% based on (c), or 19.1% based on formaldehyde (Note 21). Degradation studies indicate that there is no isotopic rearrangement (Note 22).

1,2,3-Propanetriol-2-C^{14} may be prepared by these same procedures, starting with nitromethane-C^{14}.

B. Notes

1. Quantities are chosen such as to yield 6 g. of glycerol without the addition of carrier at any stage. The procedure involves only adaptations and modifications of known methods, exclusive of the last step. The glycerol-1-C^{14} is prepared from either sodium cyanide-C^{14} or bromoacetic-1-C^{14} acid, but for convenience of illustration the two methods are combined.

2. The solution is prepared by heating the components under reflux for 15 hours in a 500-ml. three-necked flask fitted with a reflux condenser, a calcium chloride guard tube, a mercury-sealed stirrer and dropping funnel.

3. The ether layer contains nothing but solvent.

4. The dissolved salts must be removed if the product is to be distilled. A mixture of Amberlite IR-120 and IR-4B, or IR-400, is satisfactory.

5. The apparatus used was tested with nonisotopic glycerol to assure that any carry-over of product was negligible.

6. The assay is carried out by the method of Reeves,[4,5] in which the formaldehyde, formed by periodic acid oxidation of terminal carbinol groups, is collected and weighed as the dimedon derivative. The formic acid, representing C-2, appears to have a specific activity of 0.4–2.5% of that of the glycerol-1-C^{14}. The product may be mixed with pure carrier and distilled, b.p. 140–145° (1–2 mm.), with 90–95% recovery (95.5–97.5% purity).

7. Degradation shows that the formaldehyde, representing C-1, appears to have a specific activity of 1.2–1.8% of that of the glycerol-2-C^{14}. This may be due to contamination of the product, or to inadequate selectivity of the degradation method.

Doerschuk[5] has degraded specially purified glycerol-1-C^{14} by both periodate[4] and lead tetraacetate[6] oxidations. The two 1,2-glycol-cleaving reagents incorporated 0.25% and 4.2% of the original radioactivity, respectively, into the formic acid formed. All compounds were assayed in the form of barium carbonate. Mercuric oxide converts formic acid, but not formaldehyde,[7] to carbon dioxide, whereas excess lead tetraacetate oxidizes formic acid to carbon dioxide. In all cases of lead tetraacetate oxidation, the high yields of carbon dioxide obtained could explain the 4.2% radioactivity incorporation by further oxidation of some of the formaldehyde. No. rearrangement of an intermediate, or oxidation-reduction exchange between reaction products, need be postulated in the case of either oxidant. Periodate degradation is preferable.

8. The preparation of the acid is very similar to the procedure of Sallach; see calcium glycerate-1-C^{14}.

9. The initial and final pH are 10.7 and 10.0, respectively. The course of cyanide disappearance, determined in trial runs under these conditions, was 93.6% in 26 hours and 92.0% in 45.5 hours. The cyanide was assayed by titration, a correction being applied from blank runs in which no glycolaldehyde was present. A substantial quantity of unreacted activity was recovered in the isotopic runs.

10. The carrier is prepared, and purified in this same manner, from glyceric acid, obtained by the oxidation[8] of glycerol.

11. Three preliminary experiments have given yields of 30%. The product (without carrier) has a saponification equivalent of 134.5 (calc. 134.1).

12. The colorless, odorless, viscous, liquid product, from a large-scale carrier-free preliminary synthesis, was inert to alkali under the conditions of a saponification equivalent determination, and the refractive index was very close to that of an authentic sample.

The molar specific activity of the glyceryl-1-C^{14} benzoate,[9] m.p. 72° after recrystallization from ligroin, agrees with that of the glycerol-1-C^{14}.

13. The preparation is according to the procedure of Schmidt and Wilkendorf,[10] who realized a yield of 91%. See sodium 2-nitro-1,3-propanediol-2-C^{14}.

14. With pure nonisotopic paraformaldehyde the reaction began at once, and the solution became clear within 10 minutes.

15. The salt contains two molecules of methanol of crystallization.

16. The hydrogenation equipment is similar to the Parr apparatus.

17. The extremely hygroscopic salt may be recrystallized from absolute ethanol and dried at 70° (over phosphorus pentoxide, m.p. 101–103° (in an evacuated tube).

18. The melting point of a purified, nonisotopic sample is 125–127°.

19. The pH changes from 3.5 to 5.9, and the odor of methyl hexanoate is immediately apparent.

20. The colloidal particles are removed by filtration with charcoal-Hyflo.

21. A similar preparation having a saponification value of 11, equivalent to 3.8% monohexanoin, was characterized by conversion to tripalmitin, which agreed in yield and properties with that prepared from an authentic pure sample.

22. The glycerol is oxidized with periodic acid, and the formaldehyde-C^{14} formed is assayed as the dimedon derivative; the molar specific activity was 98.3% of that which was formed directly from the paraformaldehyde-C^{14} (see Note 7).

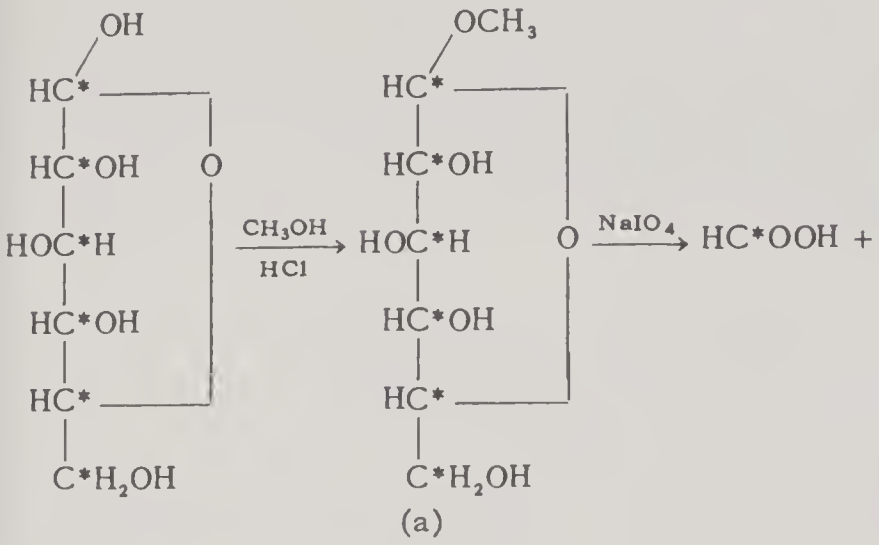
C. Other Preparations

Glycerol-1-C^{14} has been prepared[11] in 60% over-all yield from sodium cyanide, by a cyanohydrin reaction with benzyloxyacetaldehyde, followed by acetylation, giving 2-acetoxy-3-benzyloxypropionitrile-1-C^{14}. Hydrolysis of the corresponding imidic acid hydrochloride yielded ethyl 2-acetoxy-3-benzyloxypropionate-1-C^{14}, which was reduced with lithium aluminum hydride to 1-benzyloxy-2,3-propanediol-3-C^{14}, and converted to the end product by hydrogenolysis.

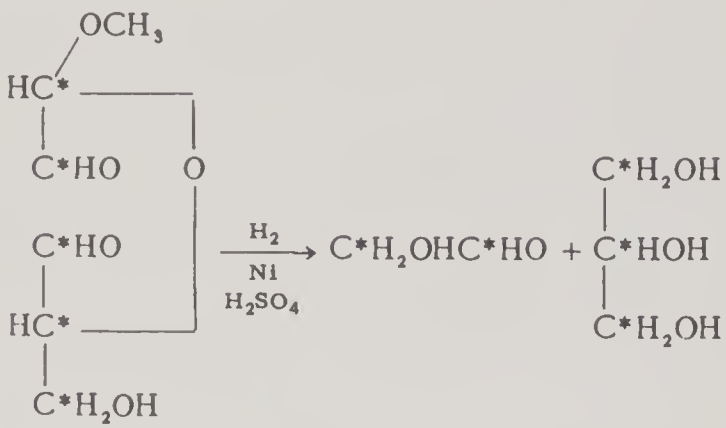
1,3-Dichloro-2-propanone, prepared in 71.8% yield from chloroacetyl chloride by the Arndt-Eistert procedure, has been reduced with lithium aluminum hydride,[12] forming 1,3-dichloro-2-propanol in 77% yield. Hydrolysis of this intermediate gave glycerol in the usual yield.

[1]O. Dimroth and R. Schweizer, Ber., *56*, 1375 (1923).
[2]R. F. Nystrom and W. G. Brown, J. Am. Chem. Soc., *69*, 1197, 2548 (1947).
[3]L. Hough, Nature, *165*, 400 (1950).
[4]R. E. Reeves, J. Am. Chem. Soc., *63*, 1476 (1941); *Organic Reactions*, Vol. II, Wiley, New York, 1944, p. 341.
[5]A. P. Doerschuk, J. Am. Chem. Soc., *73*, 5453 (1951).
[6]J. M. Grosheintz, J. Am. Chem. Soc., *61*, 3379 (1939).
[7]Y. J. Topper and H. B. Hastings, J. Biol. Chem., *179*, 1255 (1949).
[8]*Beilstein*, 4th Ed., III, p. 395.
[9]A. Fairbourne and G. E. Foster, J. Chem. Soc., *127*, 2759 (1925); S. Abraham, J. Am. Chem. Soc., *74*, 6098 (1952).
[10]E. Schmidt and R. Wilkendorf, Ber., *52*, 389 (1919).
[11]U. S. Testing Co., Chem. Eng. News, *30*, 1872 (1952).
[12]H. Schlenk and B. Lamp, J. Am. Chem. Soc., *73*, 5493 (1951).

GLYCEROL-C_3^{14}



(a)



(b) (c)

S. Abraham, J. Am. Chem. Soc., 74, 6098 (1952).

A. Procedure (Note 1)

(a) *Methyl-D-glucopyranoside-*C_6^{14}. A solution of 1.0 g. of D-glucose-C_6^{14},[1] in 10 ml. of absolute methanol which is 0.083 *N* in hydrogen chloride, is treated according to the method of Fischer,[2,3] which follows. The yield of mixed isomers is 0.91 g., 84% (Note 2).

The mixture is refluxed for 30–60 minutes. The pale yellow solution of the glucose dimethylacetal is heated for 50 hours at 100° in a sealed tube, then decolorized with charcoal and concentrated to one-third volume. The mixture is seeded and stored overnight in a refrigerator (Note 3), then is covered with ether and stored for several days in the cold, with one change of ether. The crystalline product is collected and dried at 50°.

(b) *α-D-Hydroxymethyl-C^{14}-α′-L-methoxydiglycolaldehyde-C_4^{14}*, [*D′L′-Methoxy-D-hydroxymethyldiglycolic-C_5^{14} Aldehyde*] (Note 4). A solution of the methylglucoside-C_6^{14} in 35 ml. of ice-cold water is treated with 35 ml. of 0.37 *M* sodium periodate and stored at 2° for 24 hours. The solution is titrated to the end point of methyl red; the yield is quantitative (Note 5). The solution is treated with 1.6 g. of barium chloride, and the precipitate is separated and washed with water. The filtrate is concentrated under reduced pressure below 60°, and the additional precipitate is removed.

(c) *Glycerol-C_3^{14}*. The dialdehyde is hydrogenated for 18 hours in aqueous solution over Raney nickel at 2700 p.s.i. and 140° (Note 2). The filtered solution is acidified with 3.5 ml. of concentrated sulfuric acid and refluxed for 3 hours. The solution is treated with 42 ml. of a 2,4-dinitrophenylhydrazine solution (Note 6), and the precipitate is collected, washed and dried (Note 7). The excess reagent is precipitated from the filtrate by treatment with 0.7 ml. of 38% formaldehyde solution. The filtrate is neutralized with excess barium carbonate, and the insoluble sulfate is filtered off and washed with water. The solution is decolorized with charcoal and evaporated to dryness under vacuum. The residue is extracted with hot anhydrous acetone, and the product is obtained by evaporation; yield 0.41 g., 64% based on glucose (Note 8). Two-dimensional paper chromatography shows the presence of only one isotopic compound (Note 9), and degradation studies[4] show that the distribution of isotope is uniform.

B. Notes

1. The method is based upon a modification of the degradation of glucose described by Wood[3] and Jackson.[5]

2. The product gives a negative reaction with Benedict's solution.

3. The α-methylglucoside is the first to crystallize (45%); a further yield of the α-form may be obtained by treating the mother liquor with methanolic hydrogen chloride and heating for 40 hours.

4. The nomenclature is that employed by Jackson,[5] the prefixes D′ and L′ distinguishing the configuration of C-1 in the products from the α- and β-methyl glycosides.

5. The yield is based on the titration of the formic-C^{14} acid formed from C-3.[3,5]

6. The reagent consists of 4 g. of 2,4-dinitrophenylhydrazine, 20 ml. of concentrated sulfuric acid, 30 ml. of water and 100 ml. of 95% ethanol.

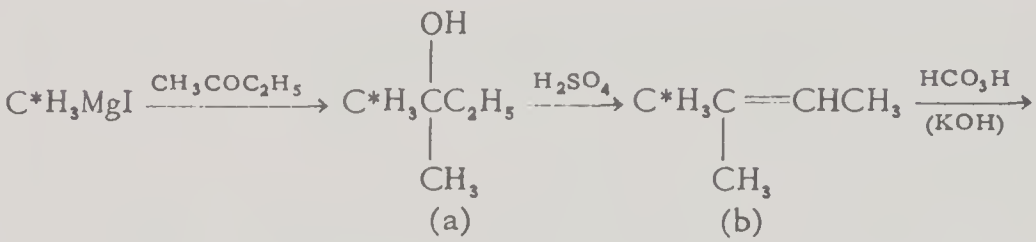
7. The yield of glycolaldehyde-C_2^{14} 2,4-dinitrophenylhydrazone is 1.01 g. (89.5%).

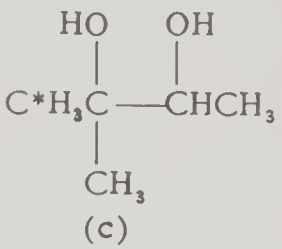
8. The yield is corrected for water content on the basis of refractive index.[6] Directions are given by Abraham for preparing glyceryl benzoate, m.p. 74–75°.

9. The solvent systems used are phenol-water and butanol-acetic acid-water. The color spray is lead tetraacetate in benzene.

[1]E. W. Putman, W. Z. Hassid, G. Krotkov and H. A. Barker, J. Biol. Chem., *173*, 785 (1948).
[2]E. Fischer, Ber., *28*, 1145 (1895).
[3]H. G. Wood, N. Lifson and V. Lorber, J. Biol. Chem., *159*, 475 (1945).
[4]D. Kritchevsky and S. Abraham, Arch. Biochem. Biophys., *39*, 305 (1952).
[5]E. L. Jackson and C. S. Hudson, J. Am. Chem. Soc., *59*, 994 (1937).
[6]*International Critical Tables*, Vol. 7, McGraw-Hill, New York, 1930, p. 68.

2-METHYL-2,3-BUTANEDIOL-1-C^{14}





J. D. Roberts, R. E. McMahon and J. S. Hine, J. Am. Chem. Soc., *72*, 4237 (1950).

A. Procedure

(a) *2-Methyl-2-butanol-1-C^{14}*. A solution of methylmagnesium-C^{14} iodide is prepared from 43.5 g. (0.31 mole) of methyl-C^{14} iodide and 7.5 g. (0.31 mole) of magnesium in 100 ml. of absolute ether under a nitrogen atmosphere. A solution of 22.4 g. (0.31 mole) of 2-butanone in 100 ml. of ether is added dropwise, and the mixture is allowed to stand overnight, then is hydrolyzed with a solution of 35 g. of ammonium chloride in 215 ml. of water. The product is extracted continuously with ether, and the extract is dried over calcium sulfate and fractionated through a 50-cm. glass helix-packed column, using 20 ml. of dry xylene as a distillation base; yield 20.1 g. (75%), b.p. 96–100° (720 mm.).

(b) *2-Methyl-2-butene-1-C^{14}*. The olefin is formed by dehydration of 2-methyl-2-butanol-1-C^{14} by the procedure described for the preparation of 2-methyl-2-butene-2-C^{14}. Xylene is used as a distillation base.

(c) *2-Methyl-2,3-butanediol-1-C^{14}*. The glycol is formed by hydroxylation of 2-methyl-2-butene-1-C^{14} by the procedure described for the 2-C^{14}-

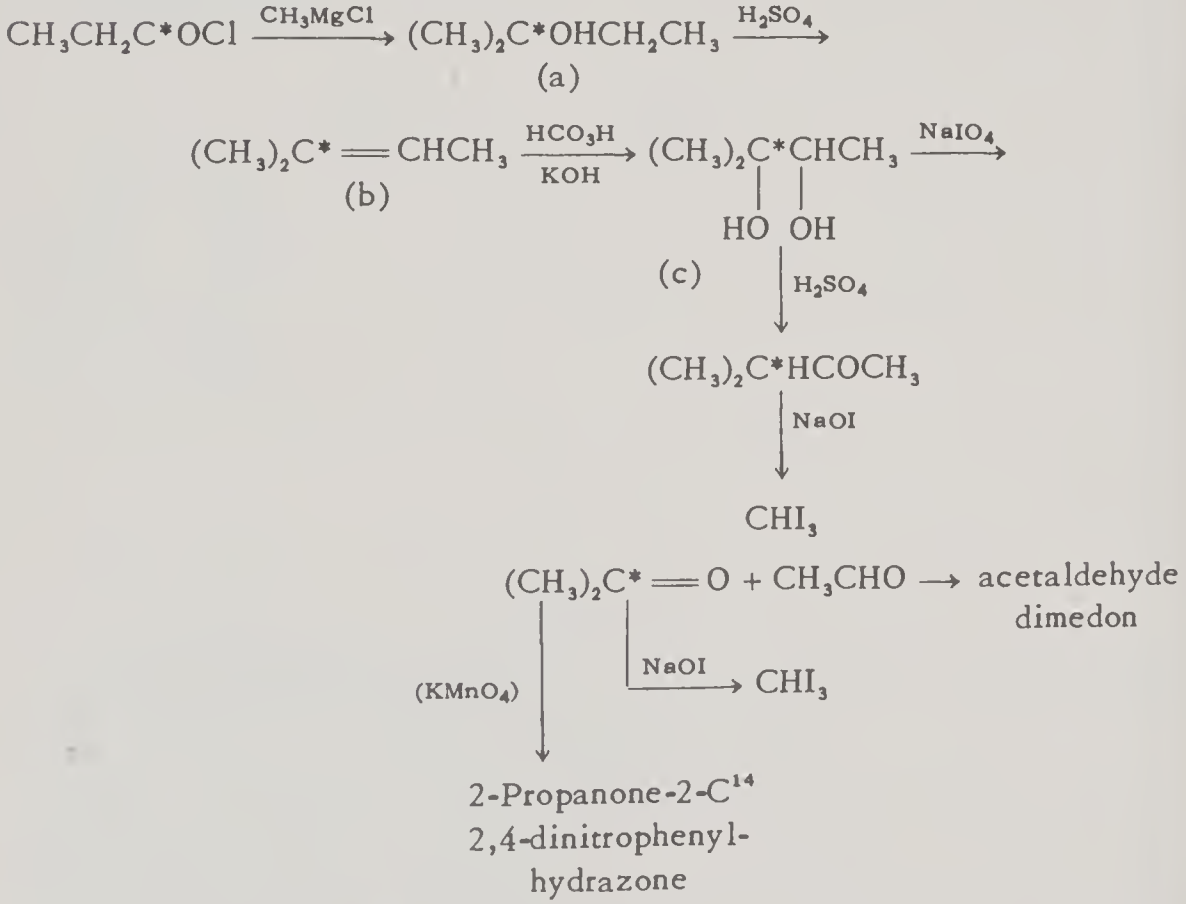
isomer. Degradation studies indicate, likewise, that there is no carbon rearrangement; see 2-methyl-2,3-butanediol-2-C^{14}, Note 8.

B. Other Preparations

2-Methyl-2-butene-1-C^{14} has been prepared by Roberts,[1] in about 42% yield, by the dehydrohalogenation of 2-chloro-2-methylbutane-1-C^{14} by heating with water. Dehydrohalogenation with sodium ethoxide forms considerable 2-methyl-1-butene.

[1] J. D. Roberts, R. E. McMahon and J. S. Hine, J. Am. Chem. Soc., *72*, 4237 (1950).

2-METHYL-2,3-BUTANEDIOL-2-C^{14}



J. D. Roberts, R. E. McMahon and J. S. Hine, J. Am. Chem. Soc., *72*, 4237 (1950).

A. Procedure

(a) *2-Methyl-2-butanol-2-C^{14}.* Propionyl-1-C^{14} chloride (20 g., 0.22 mole) in 100 ml. of absolute ether is added dropwise to a stirred, ice-cooled solution of methylmagnesium chloride prepared from 17.1 g. of magnesium in 900 ml. of ether under nitrogen. The mixture is stirred for several hours and allowed to stand overnight, and the Grignard complex

is decomposed by addition of 130 ml. of saturated ammonium chloride solution. The solid cake is separated and washed several times with ether. The combined ether extract is dried over calcium sulfate and distilled through a 50-cm. glass helix-packed column (Note 1); yield 16.1 g. (81%), b.p. 100–102° (750 mm.).

(b) *2-Methyl-2-butene-2-C*^{14} (Note 2). A mixture of 11.1 g. of 2-methyl-2-butanol-2-C^{14} and 35 ml. of 18 *N* sulfuric acid, contained in a flask equipped with a short Vigreux column, is heated in an oil-bath at 140° until distillation ceases, the distillate being collected in a Dry Ice-cooled flask containing 2 g. of potassium hydroxide. The product is distilled at reduced pressure into a flask containing 10 ml. of isoöctane, and the mixture is fractionated (Note 3) through the 50-cm. glass helix-packed column; yield 5.2 g. (59%), b.p. 36–37° (740 mm.).

(c) *2-Methyl-2,3-butanediol-2-C*^{14} (Note 4). The olefin hydroxylation is run in a 200-ml. three-necked flask equipped with: a dropping funnel, a magnetic stirrer, a reflux condenser (Note 5) surmounted by a Dry Ice-cooled condenser and a thermometer which dips below the liquid level. 2-Methyl-2-butene-2-C^{14} (2.0 g.) is added dropwise to a stirred mixture of 25 ml. of 87% formic acid and 5 ml. of 35% hydrogen peroxide at a slow enough rate to keep the temperature below 30°. When addition is complete, the mixture is stirred at room temperature for several hours. Most of the formic acid is removed by distillation, and the residue is refluxed for 30 minutes with a solution of 10 g. of potassium hydroxide in 35 ml. of water (Note 6). The mixture is saturated with potassium carbonate and extracted continuously with ether. The extract is dried over magnesium sulfate and concentrated, and the residue is mixed with 5 ml. of benzene to remove any remaining traces of moisture by azeotropic distillation. The residual glycol is collected by distillation under reduced pressure; yield 1.8 g. (61%), b.p. 96–97° (30 mm.), n_D^{25} 1.4360 (Note 7). Degradation studies (Note 8) indicate that there is no carbon rearrangement.

B. Notes

1. Xylene is used as a distillation base.

2. The procedure is a modification of the method of Norris.[1] It is demonstrated, below, that the reaction gives no carbon rearrangement.

3. The forerun (1.2 g.), b.p. 30–36°, is probably 2-methyl-1-butene-2-C^{14}.

4. The alkene hydroxylation with performic acid is based on the method of Swern.[2]

5. The reflux condenser is ice water-cooled.

6. The hydrolysis is required to decompose the hydroxy formoxy intermediate.[1]

7. Suter and Zook[3] report b.p. 75–76° (14 mm.), n_D^{25} 1.4375.

8. Directions are given by Roberts for degradation of the glycol. Sodium metaperiodate cleavage of a portion of the glycol forms a mixture of acetone and acetaldehyde which is collected by distillation. An aliquot is converted to the acetaldehyde dimedon, m.p. 142–143°, for assay. The remainder is oxidized with potassium permanganate, and one aliquot of the solution is converted to acetone 2,4-dinitrophenylhydrazone, m.p. 123–124°, for assay. The remaining solution is oxidized with alkaline iodine-potassium iodide solution to iodoform, m.p. 118–121° (dec.), for assay. C-4 is isolated by a pinacol-type rearrangement of the glycol by distillation with dilute sulfuric acid, forming 3-methyl-2-butanone-3-C^{14}, which is converted to iodoform by oxidation with alkaline iodine-potassium iodide. That the rearrangement proceeds exclusively with hydrogen (not methyl) shift is indicated by the fact that degradation of 2-methyl-2,3-butanediol-1-C^{14}, in this manner, gave rise to completely inactive iodoform.

C. Other Preparations

2-Methyl-2-butene-2-C^{14} has been prepared in about 42% yield by the dehydrohalogenation of 2-chloro-2-methylbutane-2-C^{14} by heating with water;[4] dehydrohalogenation with sodium ethoxide yields considerable amounts of 2-methyl-1-butene.

[1]J. F. Norris and J. M. Joubert, J. Am. Chem. Soc., *49*, 884 (1927).

[2]D. Swern, G. N. Billen, T. W. Findley and J. T. Scanlan, J. Am. Chem. Soc., *67*, 1786 (1945); *68*, 1504 (1946).

[3]C. M. Suter and H. D. Zook, J. Am. Chem. Soc., *66*, 738 (1944).

[4]J. D. Roberts, R. E. McMahon and J. S. Hine, J. Am. Chem. Soc., *72*, 4237 (1950).

2-AMINOETHANOL-2-C^{14} HYDROCHLORIDE

$$NaC^*N \xrightleftharpoons{HCHO} HOCH_2C^*N \xrightarrow[NaOH]{C_6H_5COCl} C_6H_5COOCH_2C^*N \xrightarrow[2.\ HCl]{1.\ LiAlH_4} \underset{(a)}{} \quad HOCH_2C^*H_2NH_2\cdot HCl \quad (b)$$

D. E. Douglas and A. M. Burditt, Can. J. Chem., *33*, 1183 (1955).

A. Procedure

(a) *Cyano-C^{14}-methyl Benzoate* (Note 1). To a stirred ice-cooled solution of 0.154 g. (2.9 mmoles) of sodium cyanide-C^{14} in 0.5 ml. of water is added 0.24 ml. of 37% formalin (2.7 mmoles). After 1 hour, 0.30 ml. (2.6 mmoles) of benzoyl chloride is added to the cold solution of gly-

colonitrile-1-C^{14} and sodium hydroxide, and stirring is continued for 3 hours. The mixture is treated with 3 ml. of 5% sodium bicarbonate and continuously extracted with ether for 2–3 hours (Note 2). The extract is dried over calcium chloride, then over Drierite.

(b) *2-Aminoethanol-2-C^{14} Hydrochloride.* The dried etheral solution of cyano-C^{14}-methyl benzoate is added slowly to 3.5 ml. of ether containing 9.1 mmoles of lithium aluminum hydride. The stirred mixture is decomposed by the slow addition of 30 ml. of water, then concentrated to remove ether, and saturated with carbon dioxide. The aluminum hydroxide is separated by centrifuging and thoroughly washed with hot water. The combined solutions are treated with 1 ml. of butyl cellosolve and 5 ml. of 0.5 *N* sodium hydroxide. The product and water are removed by lyophilization and condensed in a trap cooled with Dry Ice-isopropyl alcohol. The residue is dissolved in water, 1 ml. of the butyl cellosolve is added, and lyophilization is repeated for a total of four times. The distillate is acidified with hydrochloric acid and evaporated to dryness, and the residual product is recrystallized from isopropyl alcohol-ether; yield 0.0946 g., 30.9% based on sodium cyanide (Note 3). Paper chromatography and radioautography indicate that the product is free from isotopic impurities (Note 4).

B. Notes

1. The procedure is an adaptation of that of Aloy[1], and yields only primary amine. An aqueous solution of formaldehyde and sodium cyanide reacts like sodium hydroxide and glycolonitrile.

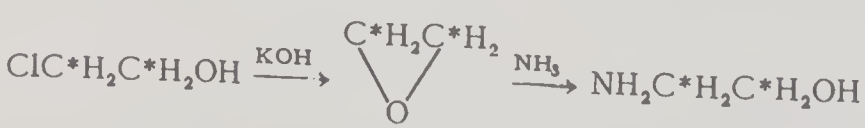
2. The aqueous solution contains 37% of the initial radioactivity.

3. A sample, after further recrystallization, melts at 79.8–82° and does not depress the melting point of an authentic sample.

4. The chromatogram is developed with water-saturated phenol, R_f 0.76.

[1] J. Aloy and C. Rabaut, Bull. soc. chim., (4) *13*, 457 (1913).

2-AMINOETHANOL-C_2^{14} HYDROCHLORIDE
(Ethanolamine-C_2^{14} Hydrochloride)



L. O. Pilgeram, E. M. Gal, E. N. Sassenrath and D. M. Greenberg, J. Biol. Chem., *204*, 367 (1953).

A. Procedure (Note 1)

To 2 ml. of an aqueous solution of 2-chloroethanol-C_2^{14}, prepared from 0.257 g. of barium carbonate-C^{14} (see ethanol-C_2^{14}) and frozen out in a 30-ml. pressure flask with liquid nitrogen, is added 6 g. of dry, powdered potassium hydroxide through a wide-bore stopcock. The cooled flask is attached to a manifold connected to a similar flask containing 10 ml. of frozen concentrated ammonium hydroxide (Note 2). The manifold is evacuated (< 1 μ) and isolated from the pump; then the liquid nitrogen around the chlorohydrin flask is replaced by a Dry Ice-methanol-bath and, five minutes later, by an ice-bath. The ethylene-C_2^{14} oxide (Note 3) evolved from the melting mixture is distilled into the flask containing the frozen ammonium hydroxide, and distillation is continued with gentle warming until a large amount of the water is collected (Note 4). The receiver flask is closed off and warmed to room temperature with shaking, then stored at 60° for 3 hours. The mixture is transferred into a beaker with 25 ml. of water and left open to the air. The solution is transferred into a flask fitted with a long Vigreux column, and 15 ml. of distillate is collected to remove ammonia (Note 5). The residual solution is acidified with 1.5 *N* hydrochloric acid and evaporated to dryness under reduced pressure in a water-bath. The yield of crude product is 0.053 g. (Note 6). Purification is accomplished by chromatography in 1.5 *N* hydrochloric acid on a 2.4 × 100-cm. column of Dowex-50 cation exchange resin (Note 7). Aliquots (20 μl.) of 5-ml. fractions of the eluate are evaporated on planchets and counted, and the activity is plotted against fraction number. All fractions belonging to a given peak are combined and identified by one-dimensional paper chromatography (Note 8). The yield of purified product is 0.0305 g., 47.5% based on barium carbonate (Note 9).

B. Notes

1. The procedure is adapted from the method of Knorr.[1]

2. The yield and type of amine produced is a function of the concentration of ammonium hydroxide. The monoethanolamine is formed in 80–90% yield, when the ammonia is in 50-mole excess. The di- and triethanolamines are formed at the expense of the mono-amine, when the ammonia excess falls below 20 moles.

3. The over-all yield of ethylene-C_2^{14} oxide is 55–70% based on barium carbonate.

4. The residue, consisting mainly of ethylene-C_2^{14} glycol, is saved for recovery of isotope.

5. The loss of radioactivity is less than 5%.

6. An alternate method of isolation involves acidification of the contents of the beaker, evaporation to dryness and extraction of the product from ammonium chloride with hot isopropyl alcohol.

7. The preparation and operation of the column is described by Shulgin.[2]

8. Whatman paper No. 4, and 1-butanol-acetic acid-water (100:21:50) are used. Ethanolamine develops a color when the paper is sprayed with 2% ninhydrin solution and heated; $R_f = 0.46$. Diethanolamine develops a faint trace of color under test tube conditions, but none on paper.

9. The over-all yield of a warm run was 60%.

[1]L. Knorr, Ber., *30*, 909 (1897).

[2]A. T. Shulgin, O. G. Lien, Jr., E. M. Gal and D. M. Greenberg, J. Am. Chem. Soc., *74*, 2427 (1952).

2-AMINOETHANOL-1-$C^{13}_{1/1}$-2-$C^{14}_{1/1}$ HYDROCHLORIDE

$$HCl \cdot NH_2C^*H_2C^\circ OOC_2H_5 \xrightarrow[CHCl_3]{NH_3} \underset{\displaystyle NH_2}{\overset{\displaystyle |}{C^*H_2C^\circ OOC_2H_5}} \xrightarrow[(HCl)]{LiAlH_4} \underset{\displaystyle NH_2 \cdot HCl}{\overset{\displaystyle |}{C^*H_2C^\circ H_2OH}}$$

A. Weissbach and D. B. Sprinson, J. Biol. Chem., *203*, 1031 (1953).

A. Procedure (Note 1)

To a stirred, ice-cooled mixture of 0.600 g. of glycine-1-$C^{13}_{1/1}$-2-$C^{14}_{1/1}$ ethyl ester hydrochloride and 0.8 ml. of chloroform is added dropwise 7 ml. of 2% ammonia in chloroform (Note 2). After 15 minutes of stirring in the cold, the precipitate is separated by centrifuging and washed with two portions of cold chloroform. The combined supernatant solutions are evaporated to dryness under vacuum; the residual glycine-1-$C^{13}_{1/1}$-2-$C^{14}_{1/1}$ ethyl ester is taken up in a few ml. of absolute ether and added dropwise to a stirred solution of 0.420 g. of lithium aluminum hydride in 5 ml. of ether. After 15 minutes, 3 ml. of water is added dropwise; the mixture is diluted with 15 ml. of water and continuously extracted (48 hr.) with ether containing 3 ml. of 20% hydrochloric acid. The extract is evaporated to dryness, and the crude product is recrystallized from a minimal volume of hot absolute ethanol by dilution with absolute ether; yield 0.200 g. (50%), m.p. 74–76° (Note 3).

B. Notes

1. The procedure is that described by Hillman[1] for preparing the free esters of amino acids from their hydrochlorides, and an adaptation of the method described by Karrer[2] for reducing 2-aminocarboxylic esters with lithium aluminum hydride.

2. The solubility of ammonia in chloroform is 2.15 g. per 100 g. at 19°.

3. Pilgeram[3] abandoned attempts to prepare singly labeled ethanolamine by the reduction of phthalylglycyl chloride, phthalylglycine ethyl ester and glycine ethyl ester with lithium aluminum hydride, because of low yields.

C. Other Preparations

2-Aminoethanol-1-$C^{13}_{1/1}$-2-$C^{14}_{1/1}$-$2H^2_2$ hydrochloride is prepared in the same manner by employing lithium aluminum hydride-H^2_4 and water-H^2_2.

2-Aminoethanol-$C^{14}_{1/2}$ hydrochloride has been prepared[4] from glycine-$C^{14}_{1/2}$ by the same procedure.

[1]G. Hillman, Z. Naturforsch, *1*, 682 (1946); Chem. Abstracts, *43*, 7425 (1949).

[2]P. Karrer, P. Portmann and M. Suter, Helv. Chim. Acta, *31*, 1617 (1948).

[3]L. O. Pilgeram, E. M. Gal, E. N. Sassenrath and D. M. Greenberg, J. Biol. Chem., *204*, 367 (1953).

[4]I. Zabin and J. F. Mead, J. Biol. Chem., *211*, 87 (1954).

2-DIMETHYLAMINO-C^{14}_1-ETHANOL

$$CH_3NHCH_2CH_2OH \xrightarrow{2HC^*HO} CH_3N(C^*H_3)CH_2CH_2OH + HC^*OOH$$

C. Artom and M. Crowder, J. Am. Chem. Soc., *74*, 2412 (1952).

A. Procedure

A mixture of 2 ml. of a 2.5 *M* solution of 2-methylaminoethanol (5 mmoles), 1.8 ml. of a solution of formaldehyde-C^{14} (4.6 mmoles) and 0.7 ml. of alkaline borate buffer, pH 10.0, (Note 1) is refluxed for 6 hours. The mixture is chilled in an ice-bath and neutralized with 2 *N* hydrochloric acid, and the unreacted amine is destroyed by adding 10 ml. of 30% sodium nitrite and 5 ml. of glacial acetic acid. After 10 minutes, the mixture is made basic with saturated sodium hydroxide and aerated for 45 minutes to remove oxides of nitrogen. The mixture is steam-distilled (Note 2) for 30 minutes, the distillate being collected in 4 ml. of *N* hydrochloric acid. Any traces of unreacted formaldehyde-C^{14} are destroyed by adding to the distillate 1 ml. of 1% nonisotopic formaldehyde, 1 ml. of *N* iodine and a slight excess of *N* sodium hydroxide solutions. The mixture is acidified and the excess iodine is reduced with 5% sodium bisulifte solution. The mixture is concentrated to a few ml., made alkaline and again steam-distilled into 4 ml. of *N* hydrochloric acid. The chemical yield is 40.6% (1.87 mmoles based on titration), and the radiochemical yield is 41.0% (Note 3).

B. Notes

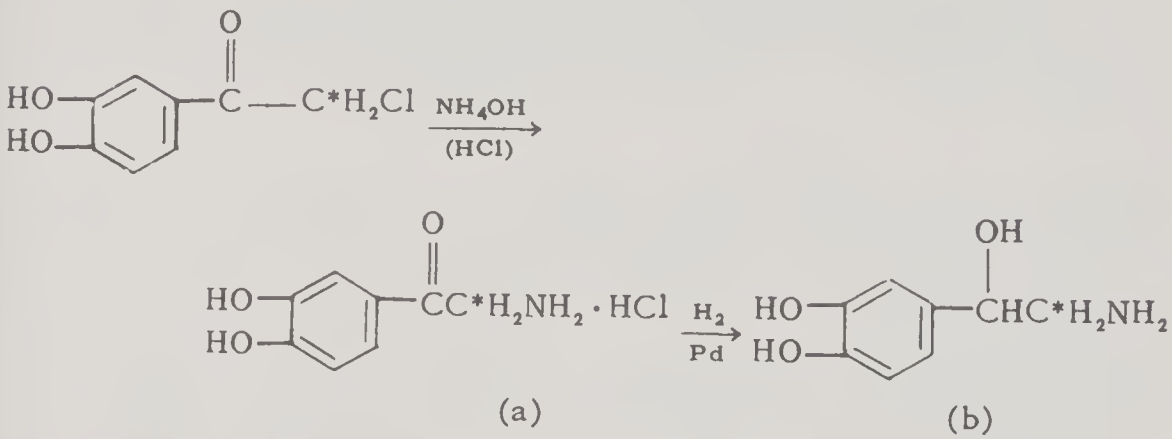
1. The stock buffer consists of 6.2 g. of boric acid in 100 ml. of *N* sodium hydroxide.

2. A recommended apparatus is that described by Parnas and Wagner.[1]

3. Investigation has shown the product to be essentially free of contamination by isotopic formaldehyde, formic acid or methanol. The picrolonate melts at 196°.

[1] J. K. Parnas and R. Wagner, Biochem. Z., *125*, 253 (1921).

α-(AMINOMETHYL-C^{14})PROTOCATECHUYL ALCOHOL (C_1^{14}-Noradrenaline)



R. W. Schayer, J. Am. Chem. Soc., *75*, 1757 (1953).

A. Procedure

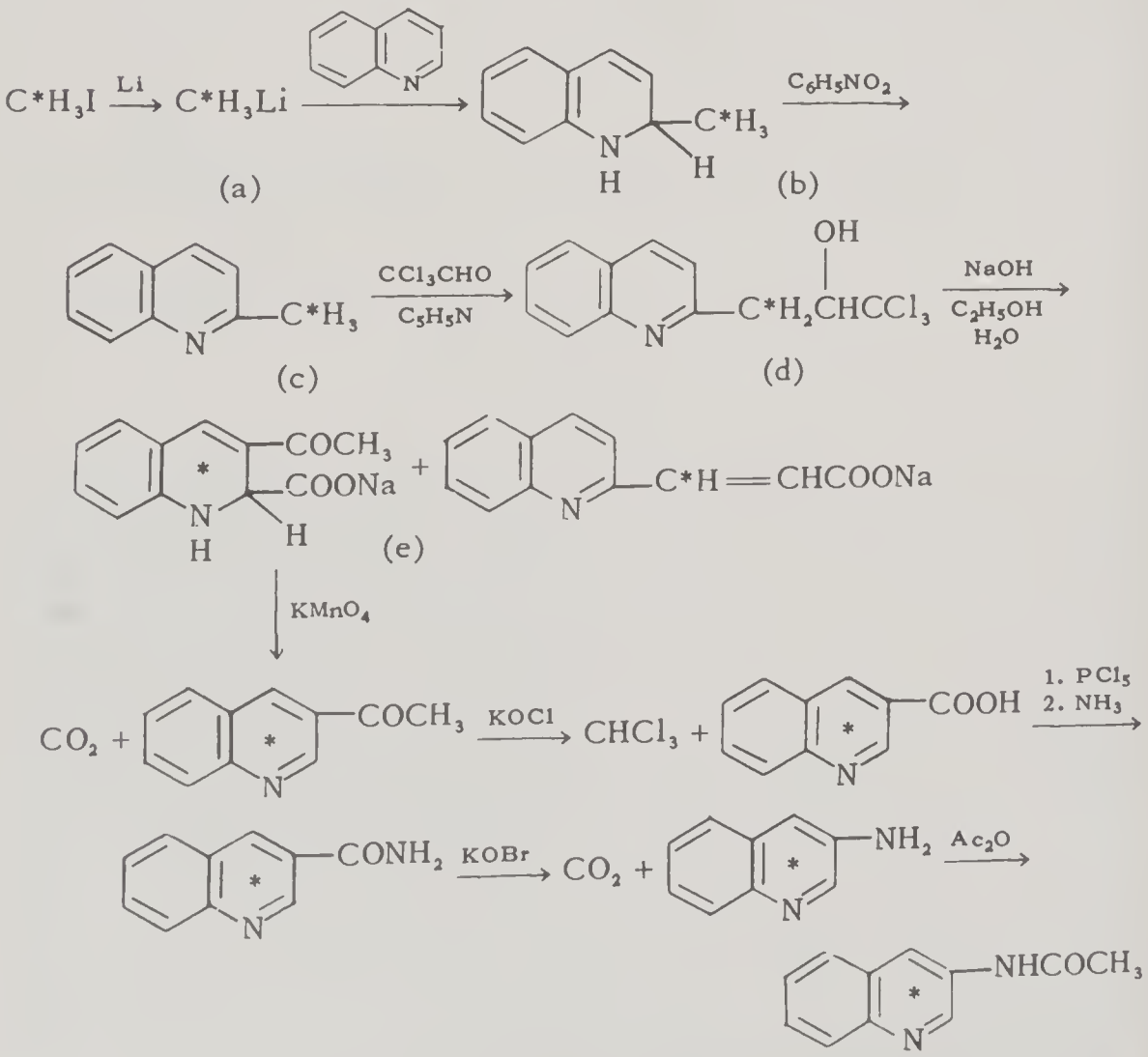
(a) *2-Amino-3′,4′-dihydroxyacetophenone-2-C^{14} Hydrochloride,* (C_1^{14}-*Noradrenalone Hydrochloride*). Ice-cold, concentrated ammonium hydroxide (0.9 ml.) is added to 0.210 g. of 2-chloro-3′,4′-dihydroxyacetophenone-2-C^{14} under hydrogen at 0°. After 10 minutes, the mixture is stored at room temperature for 20 hours with occasional shaking. The mixture is diluted under hydrogen with 0.5 ml. of absolute alcohol and cooled. The precipitate is filtered off, washed with cold 50% alcohol, alcohol, then ether, and dissolved in 3 ml. of 3 *N* hydrochloric acid. The solution is filtered and made basic, under hydrogen, with excess ammonia. The purified 2-amino-3′,4′-dihydroxyacetophenone-2-C^{14} is collected (0.093 g.) and dissolved in a minimal volume of 3 *N* hydrochloric acid. The filtered solution is diluted slowly with absolute alcohol and then ether, to crystallize the product; yield, 0.090 g. Recrystallization gives 0.077 g. of purified product.

(b) α-(*Aminomethyl-C^{14})protocatechuyl Alcohol,* [α-(*Aminomethyl-C^{14}*)-*3,4-dihydroxybenzyl Alcohol*]. The C_1^{14}-noradrenalone hydrochloride is

hydrogenated in 5 ml. of aqueous solution over 6 mg. of 5% palladium-on-aluminum oxide powder catalyst for 16 hours at 40° and under 5 p.s.i. The solution is filtered, concentrated to small volume by evaporation from the frozen state and refiltered. The combined filtrate and wash (0.5 ml.) is treated with 1 drop of ammonium hydroxide and stored in the cold under hydrogen for 4 hours. The product is collected on a filter and washed with cold water, alcohol, then ether, and dried under vacuum; yield 0.053 g., 28% based on the chloro-ketone. No isotopic impurities are detected by isotope dilution assay. A method of resolution is described by Tullar.[1]

[1]B. F. Tullar, J. Am. Chem. Soc., 70, 2067 (1948).

α-(TRICHLOROMETHYL)-2-QUINOLINEËTHANOL-β-C^{14}
(C_1^{14}-Chloralquinaldine)



W. G. Dauben and C. W. Vaughan, Jr., J. Am. Chem. Soc., 75, 4651 (1953).

A. Procedure

(a) *Methyllithium-C^{14}.* Lithium wire (22.6 g., 3.26 mmoles) is reacted with a solution of 55.15 g. (388 mmoles) of methyl-C^{14} iodide in absolute ether; yield 65% (Note 1).

(b) *1,2-Dihydro-2-methyl-C^{14}-quinoline.* To the stirred solution of methyllithium-C^{14} is added a solution of 35.03 g. (272 mmoles) of quinoline in 100 ml. of absolute ether during 1 hour. The mixture is hydrolyzed with 200 ml. of water, and the ether extract is separated, dried over magnesium sulfate and evaporated.

(c) *2-Methyl-C^{14}-quinoline, (Quinaldine-α-C^{14}).*[8] The crude 1,2-dihydro-2-methyl-C^{14}-quinoline is refluxed with 178 g. of nitrobenzene for 20 minutes, the water being removed as formed. The mixture is diluted with 200 ml. of ether and extracted with three 100-ml. portions of 3 *N* hydrochloric acid. The acid solution is cooled and basified with potassium hydroxide, and the product is extracted with three 200-ml. portions of ether. The ether solution is dried and fractionally distilled through a Podbielniak Helipak Column; yield 41.7 g., b.p. 116–120° (15 mm.) (Note 2).

(d) α-(*Trichloromethyl*)-*2-quinolineëthanol-β-C^{14}* (Note 3). A mixture of 48.5 g. (339 mmoles) of 2-methyl-C^{14}-quinoline, 52.5 g. (356 mmoles) of anhydrous chloral and 49.7 g. of anhydrous pyridine is heated on a steam-bath for about 2 hours (Note 4). The mixture is added to about 250 ml. of cold water, and the solid is collected on a filter, washed with water, dried and recrystallized from methylene chloride-hexane; yield 69 g. (70%), m.p. 147.1–148.0°.

(e) *Sodium 3-Acetyl-(C_1^{14}-1,2-dihydroquinaldate)* (Note 5). To a refluxing solution of 10.0 g. (34.3 mmoles) of α-(trichloromethyl)-2-quinolineëthanol-β-C^{14} in 35 ml. of 99% ethanol in a 2-l. flask is added 40 ml. of water followed by 35 g. of 23% sodium hydroxide, added as quickly as possible (Note 6). The mixture is refluxed for 5 minutes, then concentrated by evaporating one-half of the solvent under reduced pressure. The mixture is diluted with 50 ml. of 99% ethanol and cooled at −40° for several hours. The orange product is collected and air-dried (Note 7); yield 3.8 g. (38%). Degradation studies (Note 8) indicate that the isotope is located in the ring as a result of rearrangement.

B. Notes

1. The yield is determined by diluting an aliquot of the filtered solution with water and titrating with standard acid. For other preparations see 6-methyl-5-hepten-2-one-1-C^{14}.

2. The forerun is 4.24 g. The radiochemical loss due to column hold-up may be minimized by distilling 10 g. of carrier, added to the pot.

3. The procedure is that of Alberts and Bachman.[1]

4. Undesirable side reactions occur if the temperature rises above 100°.

5. The preparation is incidental to a study of the mechanism of rearrangement of chloralquinaldine with alkali.[2,3] The rearrangement product is in addition to the expected 2-quinolineacrylic-β-C^{14} acid.[4]

6. The subsequent reaction is immediate and violent.

7. The salt crystallizes as the trihydrate.

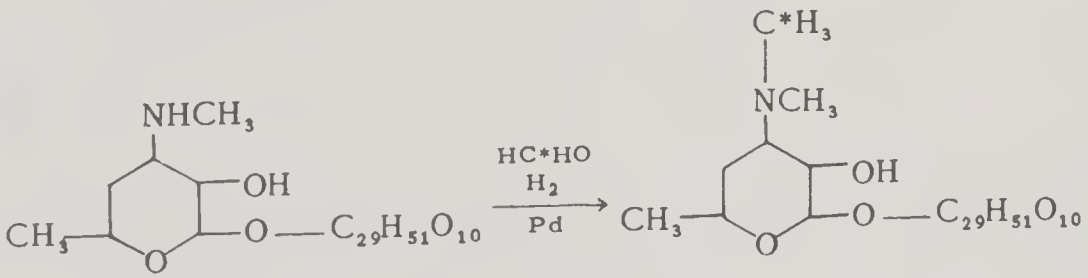
8. Directions are given by Dauben for degrading the compound by the sequence: methyl C_1^{14}-3-quinolyl ketone (yield 60%, m.p. 99.2–99.6°), (C_1^{14}-3-quinoline)carboxylic acid (yield 79%, m.p. 272–275°), (C_1^{14}-3-quinoline)carboxamide (yield 57%, m.p. 197.5–198.5°), C_1^{14}-3-aminoquinoline (yield 74%, m.p. 81–82°) and 3-acetamido(C_1^{14}-quinoline) (yield 75%, m.p. 166.2–167°). The molar specific activity of each compound is the same as that of the starting material.

C. Other Preparations

In the same manner from quinaldine-2-C^{14} are prepared: chloralquinaldine-2-C^{14}, sodium 3-acetyl-1-C^{14}-1,2-dihydroquinaldate, methyl 3-quinolyl ketone-C^{14}, 3-quinolinecarboxylic-C^{14} acid, 3-quinolinecarboxamide-C^{14} and finally carbon-C^{14} dioxide and nonisotopic 3-aminoquinoline.

[1]A. A. Alberts and G. B. Bachman, J. Am. Chem. Soc., *57*, 1284 (1935).
[2]B. R. Brown, D. L. Hammick and R. Robinson, J. Chem. Soc., *1950*, 780.
[3]R. B. Woodward and E. C. Kornfeld, J. Am. Chem. Soc., *70*, 2508 (1948).
[4]A. Einhorn, Ber., *19*, 904 (1886); A. Einhorn and P. Sherman, Ann., *287*, 38 (1895).

N-METHYL-C^{14}-de-*N*-METHYLERYTHROMYCIN (C_1^{14}-Erythromycin)



E. H. Flynn, H. W. Murphy and R. E. McMahon, J. Am. Chem. Soc., 77, 3104 (1955).

A. Procedure

A stirred mixture of 0.740 g. (1.03 mmoles) of de-*N*-methylerythromycin (Note 1), 35 ml. of absolute alcohol, 0.225 g. of palladium-black and 3 ml. of 1% aqueous formaldehyde-C^{14} (1 mmole) is hydrogenated at 1 atmosphere for 48 hours at 28°. The mixture is treated with 0.001 g. of formaldehyde and 0.050 g. of the catalyst, and hydrogenation is continued

for 24 hours. The catalyst is separated by centrifuging, and the solution is evaporated under reduced pressure. The residue is mixed with 15 ml. of water and stored at −20° for 24 hours. The solution is heated to 60°, and the crystalline product is collected and dried; yield 0.300 g. of anhydrous product (Note 2). Bioassay[1] indicates that the purity is 98%, paper chromatography indicates that 99%, of the bioactivity is associated with the product (Note 3), and radioautography indicates the presence of only one radiochemical species. An additional 0.315 g. of product is obtained by evaporating the mother liquor (Note 4).

B. Notes

1. Directions are given by Flynn for preparing the starting material by degradation of erythromycin. The reaction between erythromycin and benzyl chloroformate removes one methyl group from the dimethylamino function, and the resulting *O,N*-dicarbobenzyloxy-de-*N*-methylerythromycin (60% yield) is converted to the starting material (85% yield) by hydrogenolysis.

2. The dihydrate melts indefinitely at 135–139°, and has an X-ray pattern[2] and an infrared spectrum identical with an authentic sample.

3. About 1% of the bioactivity is associated with erythromycin B.[3]

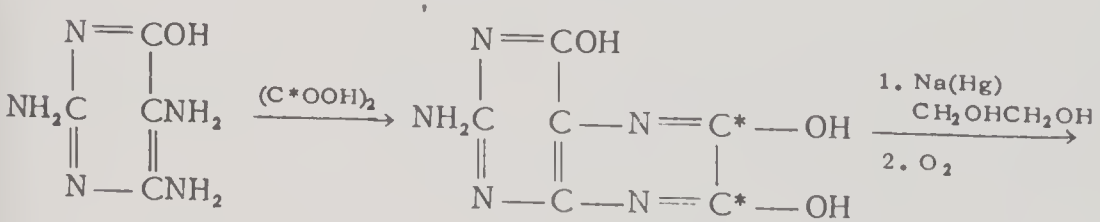
4. The second crop exhibits the same specific activity, but only 63% of the bioactivity shown by the first crop.

[1]C. Higgins, R. C. Pittenger and J. M. McGuire, Antibiotics and Chemotherapy, 3, 50 (1953).

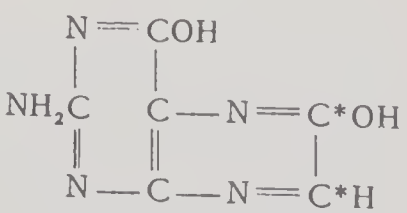
[2]H. A. Rose, Anal. Chem., *26*, 938 (1954).

[3]C. W. Pettinga, W. M. Stark and F. R. Van Abeele, J. Am. Chem. Soc., *76*, 569 (1954).

2-AMINO-4,6-PTERIDINEDIOL-6,7-C_2^{14}
(Xanthopterin-6,7-C_2^{14})



(a)



(b)

R. M. Anker and J. W. Boehne III, J. Am. Chem. Soc., *74*, 2431 (1952).

A. Procedure

(a) *2-Amino-4,6,7-pteridinetriol-6,7-C_2^{14}*, (*Leucopterin-6,7-C_2^{14}*). Oxalic-C_2^{14} acid (0.183 g., 2 mmoles) is mixed with 0.645 g. (5 mmoles) of 2,5,6-triamino-4-pyrimidinol in a Pyrex ignition tube (Note 1). The tube is sealed, after dispelling water vapor at 130°, and the temperature is raised slowly to 250° over a period of 90 minutes. The tube is cooled, the high internal pressure is released cautiously, and the product is dissolved in 30 ml. of hot 0.5 *N* sodium hydroxide. The impurities are removed by filtration (Norit), and the filtrate is poured into 30 ml. of boiling *N* hydrochloric acid and left in a refrigerator overnight. The product is collected, washed with water and dried; yield 0.234 g. (Note 2).

(b) *2-Amino-4,6-pteridinediol-6,7-C_2^{14}*. The crude leucopterin-6,7-C_2^{14} (0.233 g.), in 50-mg. lots, is treated with 2 ml. of anhydrous ethylene glycol and 0.5 g. of 5% sodium amalgam, and heated under reflux at 200°. Similar portions of amalgam are added at 30 and 60 minutes. The mixture is cooled rapidly at 90 minutes, and 5 ml. of anhydrous acetone is added, followed by the dropwise addition of a 15% solution of hydrogen chloride in absolute methanol until the layers become miscible (Note 3). The alkaline solution is separated from the mercury, adjusted to 100 ml. with dry acetone and stored in a refrigerator overnight. The precipitate is collected on a sintered glass filter, washed with acetone and dried. The sodiumdihydroxanthopterin-6,7-C_2^{14} (Note 4) is oxidized by allowing 7-ml. portions of 0.5 *N* ammonium hydroxide, in contact with air, to percolate slowly through the filter until the solid is dissolved (Note 5). The crude product is digested with hot *N* hydrochloric acid, and a red precipitate is removed by filtration of the cooled mixture. The 0.067 g. of product recovered from the filtrate is dissolved in 50 ml. of *N* ammonium hydroxide and put on an anion exchange column (Note 6). The solvent is displaced with water, and the column is eluted with 0.02 *N* ammonium chloride. Fractions are taken, and the ultraviolet absorption is measured[1] at 390, 345 and 300 mμ, after adjusting the pH to 11 with sodium hydroxide. The eluate is collected when the ratio of optical densities at 390 and 345 mμ is 3.0, and that at 390 and 300 mμ is at least 11. The yield of product from nearly 3 liters of eluate is 0.041 g. (Note 7).

B. Notes

1. A 10 × 75-mm. tube, constricted near the end, is used. The volume must be as small as possible to minimize sublimation of oxalic acid from the mixture.

2. The pale yellow solid contains a red impurity.[1] Purrmann[2] has reported a yield of 90% by the unmodified method.

3. Excess acid precipitates dihydroxanthopterin,[3] (2-amino-4,6-dihydroxydihydropteridine), and gives inferior yields.

4. This α-dihydroxanthopterin,[1,4] is not the same as the synthetic 7,8-isomer,[3] β-dihydroxanthopterin, the structure of which is known.

5. This method of oxidation is believed to be superior to the use of any other of many oxidizing agents[1] tried.

6. A 12 × 100-mm. column of 300-mesh Dowex-1, is employed in the chloride-form. Xanthopterin appears on the column as a yellow band with intense greenish-yellow fluorescence in ultraviolet light.

7. Paper chromatography with aqueous 2,4-lutidine shows the presence of only a single yellow-fluorescent band. This preparative procedure is the result of many trials. The conditions are believed to be optimal at this scale, for which the methods of Totter[5] and of Elion[1] were found to be unsatisfactory. The compound forms a monohydrate.

[1]G. B. Elion, A. E. Light and G. H. Hitchings, J. Am. Chem. Soc., *71*, 741 (1949).

[2]R. Purrmann, Ann., *544*, 182 (1940).

[3]G. H. Hitchings and G. B. Elion, J. Am. Chem. Soc., *71*, 467 (1949).

[4]B. L. O'Dell, J. M. Vandenbelt, F. S. Bloom and J. J. Pfiffner, J. Am. Chem. Soc., *69*, 250 (1947).

[5]J. R. Totter, J. Biol. Chem., *154*, 105 (1944).

2-CHLOROETHANOL-2-C^{14}

$$ClC^*H_2COOH \xrightarrow{SOCl_2} ClC^*H_2COCl \xrightarrow{LiAlH_4} ClC^*H_2CH_2OH$$
(a) (b)

H. R. V. Arnstein, Biochem. J., *48*, 27 (1951).

A. Procedure

(a) *Chloroacetyl-2-C^{14} Chloride.* The crude chloroacetic-2-C^{14} acid prepared from 2.60 g. (43.4 mmoles) of acetic-2-C^{14} acid (see chloroacetic-1-C^{14} acid) is refluxed for 5 hours with 5 ml. of thionyl chloride. The mixture is stored at room temperature for 2 days (Note 1), diluted with 53.2 mmoles of carrier chloroacetyl chloride and fractionally distilled through a 1 × 6-cm. Vigreux column, the fraction boiling at 100-106° (760 mm.) being collected (Note 2).

(b) *2-Chloroethanol-2-C^{14}* (Note 3). To a stirred solution of the chloroacetyl-2-C^{14} chloride in 50 ml. of absolute ether is added slowly a solution of 2.75 g. of lithium aluminum hydride in 50 ml. of ether. The mixture is stirred for 30 minutes, then hydrolyzed by the dropwise addition of 10% sulfuric acid. The aqueous layer is separated and extracted with several portions of ether. The combined ether extract is dried over magnesium

sulfate, concentrated and vacuum-distilled, with use of the Vigreux column; yield 1.71 g., b.p. 80–90° (15 mm.).

B. Notes

1. McMaster[1] has reported that the yield of chloroacetyl chloride is 55% after 24 hours of heating.

2. Thionyl chloride is not removed completely, but the distillate is sufficiently pure for the next step.

3. The procedure is a modification of the method of Sroog,[2] who reported a yield of 62%. Reduction of the corresponding ester and acid form the compound in yields of 37% and 13%, respectively.

C. Other Preparations

2,2,2-Triphenylethanol-1-C^{14} is prepared[3] in a similar manner. A solution of 6.15 g. (20 mmoles) of triphenylacetyl-1-C^{14} chloride in 120 ml. of absolute ether is added dropwise to a stirred solution of lithium aluminum hydride in ether (10 ml., 2 moles per liter). The mixture is refluxed for 1 hour and hydrolyzed, and the ether solution is separated and evaporated; yield 5.45 g. (97%), m.p. 112°, not altered by recrystallization from alcohol.

[1]L. McMaster and F. F. Ahmann, J. Am. Chem. Soc., *50*, 145 (1928).

[2]C. E. Sroog, C. M. Chich, F. A. Short and H. M. Woodburn, J. Am. Chem. Soc., *71*, 1710 (1949).

[3]L. Pichat and M. Audinot, Bull. soc. chim., France, *19*, 466 (1952).

1,1,1-TRICHLORO-2-METHYL-C^{14}-2-PROPANOL-3-C^{14} (C_2^{14}-Chloretone)

$$CCl_3COOC_2H_5 \xrightarrow{C^*H_3MgI} CCl_3C(C^*H_3)_2 \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad | \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad OH$$

S. S. Jackel, E. H. Mosbach, J. J. Burns and C. G. King, J. Biol. Chem., *186*, 569 (1950).

A. Procedure (Note 1)

To an ice-cooled Grignard solution, prepared from 0.876 g. (36.1 mmoles) of magnesium, 34.8 mmoles of methyl-C^{14} iodide and 27.9 ml. of absolute ether, is added a solution of 2.9 g. (15.2 mmoles) of ethyl trichloroacetate in 20 ml. of ether in 3 to 5-ml. portions. After ten minutes, the mixture is refluxed for 5 minutes, then cooled in an ice-bath and hydrolyzed with 10 ml. of saturated ammonium chloride solution. The ether is evaporated under reduced pressure at the water pump, and the

residue is steam-distilled into an ice-cooled receiver. A 4–5-ml. forerun is discarded, and the next 10 ml. of distillate is collected and set aside to crystallize. The crude product is filtered off, air-dried for 5 minutes, pressed between filter papers and sublimed at 60–70° (20–30 mm.); yield 0.346 g. (11%), m.p. 77–78° (Note 2).

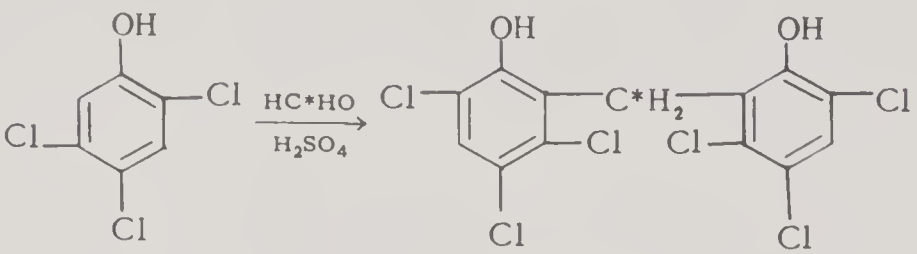
B. Notes

1. The method is that of Taffe.[1] The usual synthesis, by the alkaline condensation of acetone and chloroform, was found to give unsatisfactory yields on a small scale.

2. The product has the composition of a hemihydrate. Yields of 10–25% were obtained in trial runs. The anhydrous compound melts at 97°.

[1]M. Taffe, Roczniki Formaiji., *2*, 99 (1923); Chem. Abstracts, *18*, 2328 (1924).

2,2′-METHYLENE-C^{14}-BIS(3,4,6-TRICHLOROPHENOL) (C_1^{14}-Hexachlorophene)



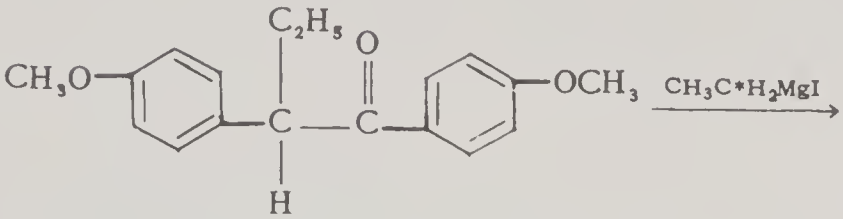
H. M. Isikow and W. S. Gump, J. Am. Chem. Soc., *74*, 2434 (1952).

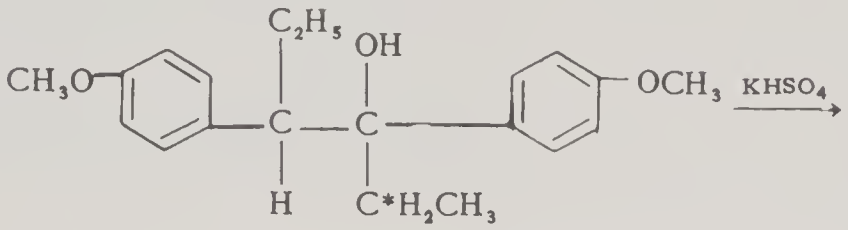
Procedure

The apparatus consists of a 200-ml. three-necked conical flask equipped with a sealed stirrer, condenser and thermometer. To a vigorously stirred mixture of 2.56 g. (14 mmoles) of 2,4,5-trichlorophenol,[1] 7 ml. of ethylene chloride and 8.7 g. of fuming sulfuric acid (20% SO_3), maintained at 60° in a water-bath, is added slowly (during 1 hour) 0.47 ml. of an aqueous methanol solution containing 3.5 mmoles of formaldehyde-C^{14}. The mixture is refluxed for 1 hour, then is cooled and treated with 50 g. of crushed ice. The solvent and excess phenol are removed by steam-distillation (3 hours), and the product is collected on a filter, washed with water and dried at 100°; yield 1.26 g., m.p. 163–166° (cor.). The crude product is decolorized and crystallized from 6 ml. of carbon tetrachloride, yield 0.672 g. (47%), m.p. 166–167° (cor.). The radiochemical yield is raised to 60% by recrystallizing 0.3 g. of carrier from the mother liquors.

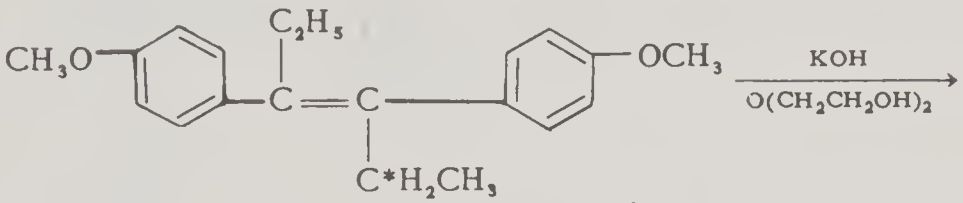
[1]E. J. Nikawitz and W. S. Gump, U. S. 2,509,245; Chem Abstracts, 44, 10735 (1950).

α-ETHYL-α′-ETHYL-1-C^{14}-4,4′-STILBENEDIOL
(C_1^{14}-Diethylstilbestrol)

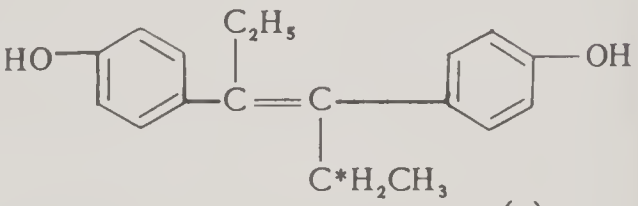




(a)



(b)



(c)

G. H. Twombly and E. F. Schoenewaldt, Cancer, *4*, 296 (1951); Nucleonics, 7 (4), 53 (1950).

A. Procedure

(a) *α-Ethyl-1-C^{14}-β-ethyl-α-(p-methoxyphenyl)-p-methoxyphenethyl Alcohol.* To a cold stirred Grignard solution, prepared from 0.312 g. (2 mmoles) of ethyl-1-C^{14} iodide, 0.0488 g. (2 mmoles) of magnesium turnings and 1 ml. of ether, is added slowly 0.290 g. (1 mmole) of 2-(*p*-methoxyphenyl)-4-methoxybutyrophenone in 1 ml. of absolute ether. The mixture is stirred at room temperature for 20 hours, then hydrolyzed with aqueous ammonium chloride solution. The ether extract is evaporated to give 0.308 g. of mixed *cis*- and *trans*-isomers, m.p. 70–100°; yield 96% based on deoxyanisoin (Note 2). The radiochemical yield is 48% (Note 3).

(b) *α-Ethyl-α′-ethyl-1-C*^{14}*-4,4′-dimethoxystilbene* (Note 4). The mixture of crude carbinols is heated with 0.150 g. of potassium acid sulfate at 200–210° for 1 hour with stirring. The product is extracted into ether, which yields a semicrystalline product upon evaporation. Recrystallization from alcohol yields 0.085 g. of well-formed plates. The combined and evaporated mother liquor, containing isomeric product, is retreated twice with potassium acid sulfate (Note 5); total yield 0.151 g. (52%), m.p. 122–123° (Note 6).

(c) *α-Ethyl-α′-ethyl-1-C*^{14}*-4,4′-stilbenediol.* The stilbestrol dimethyl ether is heated for 4 hours under nitrogen with 0.4 g. of potassium hydroxide and 2 ml. of diethylene glycol at 220–230° (Note 7). The solution is filtered and acidified, yielding a grayish-white precipitate which is collected, decolorized with charcoal and recrystallized from dilute alcohol and from benzene; yield 0.073 g., m.p. 170–170.5°, 23.3% radiochemical yield.

B. Notes

1. The procedure is a modification of the method of Dodds[1] and of Wilds,[2] who gives directions for preparing the starting material. The use of a similar procedure for an isotopic preparation has been reported by Hanahan.[3]

2. The two pure forms,[2] obtained by fractional crystallization from alcohol, melt at 114–117° and 83–83.5°, respectively.

3. The product was an oily material from which only trace amounts of crystalline material could be isolated, when an equimolar ratio of reactants was employed. The radiochemical yield might be increased, perhaps, by treating the ketone with 1 mole of isotopic Grignard solution, followed by another mole of nonisotopic reagent.

4. The dehydration is effected according to the method of Rubin,[4] as the use of *p*-toluenesulfonic acid[2] gives very poor yields.

Weygand[5] has shown by the methylation of stilbestrol with diazomethane in the presence of methanol-C^{14}, forming only nonisotopic stilbestrol dimethyl ether (81% yield), that the mechanism cannot be of the type proposed by Schönberg.[6]

5. The liquid *cis*-isomer may be rearranged to the crystalline *trans*-form by the use of iodine[2], a combination of iodine and certain metal halides,[4] or by sunlight.[1]

6. The yield[2,3] is 78–80% at the larger scale from either isomer. The product may be recrystallized from petroleum ether (b.p. 60–90°). Spectrophotometric and biological assay (modified Allen-Doisy) show that solutions in corn oil or 0.002 *N* alkali are stable for at least 30 days, when stored in a refrigerator.[3]

7. Demethylation with acids fails.[1]

C. Other Preparations

In a similar manner, starting with ethylmagnesium-2-C^{14} iodide, is prepared α-ethyl-α′-ethyl-2-C^{14}-4,4′-stilbenediol in 27% over-all yield.

[1]E. C. Dodds, L. Goldberg, W. Lawson and R. Robinson, Proc. Roy. Soc. London, *B127*, 140 (1939).

[2]A. L. Wilds and W. R. Biggerstaff, J. Am. Chem. Soc., 67, 789 (1945).

[3]D. J. Hanahan, E. G. Daskalakis, T. Edwards and H. J. Dauben, Jr., Endocrinology, *53*, 163 (1953).

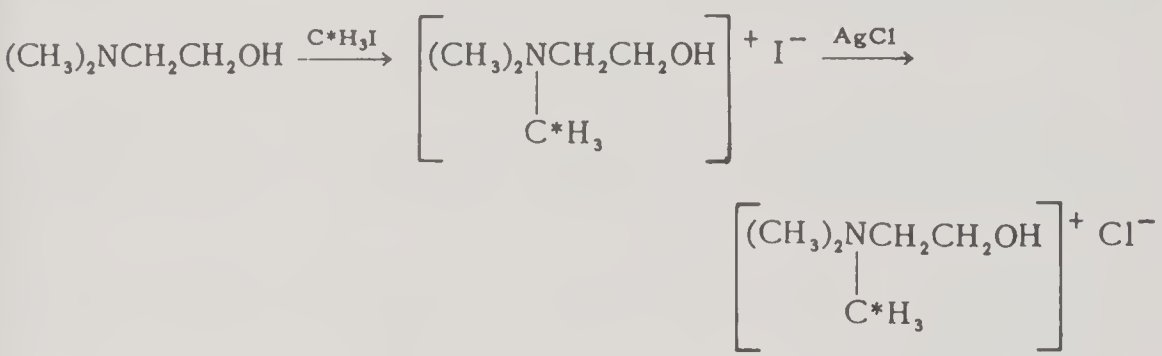
[4]M. Rubin, A. Kozlowski and M. R. Salmon, J. Am. Chem. Soc., 67, 192 (1945).

[5]F. Weygand and K. D. Kirchner, Angew. Chem., *64*, 203 (1952).

[6]A. Schönberg and A. Mustafa, J. Chem. Soc., *1946*, 746.

ONIUM COMPOUNDS

(2-HYDROXYETHYL)TRIMETHYL-C_1^{14}-AMMONIUM CHLORIDE (C_1^{14}-Choline Chloride)



M. F. Ferger and V. du Vigneaud, J. Biol. Chem., *185*, 53 (1950).

A. Procedure

The methyl-C^{14} iodide, prepared from 3.88 mmoles of methanol-C^{14}, is distilled in a stream of dry nitrogen into a solution of 0.346 g. (3.9 mmoles) of 2-dimethylaminoethanol and 5 ml. of ethanol in a trap at −60°. The mixture is stored at room temperature for 17 hours; then the precipitate is collected, and the filtrate is evaporated to dryness. Both fractions of (2-hydroxyethyl)trimethyl-C_1^{14}-ammonium iodide are recrystallized from boiling absolute ethanol, combined, dissolved in 40 ml. of absolute ethanol and shaken with a slight excess of silver chloride. The silver salts are filtered off and washed with ethanol and hot water. The combined solution is evaporated to dryness, and the crude product is recrystallized from ethanol-ether; yield 52% based on methanol-C^{14} (Note 1). The product is assayed as the chloroplatinate (Note 2).

B. Notes

1. du Vigneaud[1] has prepared an isotopic choline reineckate in 64% yield based on methanol. The reineckate ion is removed by treating the

salt in 50% aqueous acetone solution with silver sulfate. Also see Lemmon.[3a]

2. Arnstein[2] has found that wet combustion of choline may give low results due to incomplete oxidation. A sample (13 μc./mg.) stored at room temperature in the dark for 21 months in an evacuated sealed tube was found to be 63% decomposed.[3] The decomposition products from self-irradiation are only trimethylamine-C_1^{14} and acetaldehyde; experimental details are given by Tolbert.[3] The abnormal radiation sensitivity of this compound and the sensitivity of six analogs have been carefully investigated by Lemmon.[3a]

Challenger[4] has reported the preparation by the described method and assayed the product directly as the picrate.

A systematic degradation of choline is described by Elwyn.[5] The conversion to trimethylamine (50–80% yield) is described by Bregoff[6] with the aid of an apparatus diagram.

C. Other Preparations

(2-Hydroxyethyl)trimethyl-C_1^{14}-ammonium chloride has been prepared by Arnstein,[2] by essentially the procedure described. The product was degraded by oxidation with alkaline permanganate according to the method of du Vigneaud.[1] The trimethylamine-C_1^{14} formed was converted to the chloroplatinate, which is assayed best as the barium carbonate obtained by (wet) combustion.

The preparation by essentially the same method has been described by Christenson.[7] The reactants were heated in a sealed tube at 60° for 1.5 hours, then the nonvolatile residue was heated with silver oxide, and the filtered solution of quaternary base was treated with hydrochloric acid; yield 87% based on methyl iodide. The method has been used by Byerrum.[8]

The preparation by effecting the condensation in ether in a closed vessel has been reported by Winzler.[9] Roe[10] has carried out the condensation in benzene at room temperature, and converted the iodide to the chloride in the described manner; yield over 95% based on methyl iodide. Lemmon[3a] has employed methanol solution and converted the iodide by the use of silver oxide and titration with hydrochloric acid.

The preparation of choline C^{14}-acetate and its chromatography have been described by Heyndrickx.[11] A mixture of sodium C^{14}-acetate, 0.3 ml. of acetic anhydride, 0.3 ml. of acetic acid and 0.3 g. of choline chloride is refluxed for 30 minutes. The product is washed with absolute ether and dried under vacuum at 40°; yield 60%. The compound is 54% hydrolyzed after 24 hours of exposure to the atmosphere (80% relative humidity) at 20 ± 1°.

(2-Chloroethyl)trimethyl-C_1^{14}-ammonium chloride has been prepared[3a] by treating the choline chloride with thionyl chloride at room temperature.

Ethyltrimethyl-C_1^{14}-ammonium chloride has been prepared[3a] from trimethylamine-C_1^{14} and ethyl iodide, followed by conversion of the ethyltrimethyl-C_1^{14}-ammonium iodide to the chloride.

[1]V. du Vigneaud, M. Cohn, J. P. Chandler, J. R. Schenck and S. Simonds, J. Biol. Chem., *140*, 625 (1941); D. Glick, J. Biol. Chem., *156*, 643 (1944).

[2]H. R. V. Arnstein, J. Chem. Soc., *1952*, 4527.

[3]B. M. Tolbert, P. T. Adams, E. L. Bennett, A. M. Hughes, M. R. Kirk, R. M. Lemmon, R. M. Noller, R. Ostwald and M. Calvin, J. Am. Chem. Soc., *75*, 1867 (1953); R. M. Lemmon, Nucleonics, *11*,(10), 44 (1953).

[3a]R. M. Lemmon, M. A. Parsons and D. M. Chin, J. Am. Chem. Soc., 77, 4139 (1955).

[4]F. Challenger, D. B. Lisle and P. B. Dransfield, J. Chem. Soc., *1954*, 1760.

[5]D. Elwyn, A. Weissbach, S. S. Henry and D. B. Sprinson, J. Biol. Chem., *213*, 281 (1955).

[6]H. M. Bregoff and C. C. Delwiche, J. Biol. Chem., *217*, 819 (1955).

[7]F. Christenson and B. M. Tolbert, Atomic Energy Commission Report, AECU-373; Nuclear Sci. Abstracts, *3*, 1199 (1949); Nucleonics, 7 (4), 56 (1950).

[8]R. U. Byerrum and R. E. Wing, J. Biol. Chem., *205*, 637 (1953).

[9]R. J. Winzler, Nucleonics, 7 (4), 56 (1950).

[10]A. Roe, J. Elisha Mitchell Sci. Soc., *67*, 54 (1951); through Chem. Abstracts, *47*, 2126 (1953).

[11]A. Heyndrickx, J. Am. Pharm. Assoc., Sci. Ed., *42*, 680 (1953).

(2-HYDROXYETHYL-2-C^{14})TRIMETHYLAMMONIUM CHLORIDE
(Choline-α-C^{14} Chloride)

$$ClC^*H_2CH_2OH \xrightarrow{(CH_3)_3N} [(CH_3)_3NC^*H_2CH_2OH]^+ Cl^-$$

H. R. V. Arnstein, Biochem. J., *48*, 27 (1951); J. Chem. Soc., *1952*, 4527.

A. Procedure

A mixture of 1.39 g. of 2-chloroethanol-2-C^{14} and 2 ml. of trimethylamine is stored for 48 hours in a sealed tube at room temperature, then heated at 90–100° for 4 hours. The excess amine is removed by evaporation, with final heating at 215° under vacuum; yield 2.3 g. The product is assayed as the chloroplatinate (Note 1) or as the mercuric chloride complex. Degradation studies (Note 2) indicate that there is no isotopic rearrangement.

B. Notes

1. The wet combustion procedure of Van Slyke and Folch[1] is unsatisfactory for the assay of choline itself, due to incomplete oxidation. The platinum double salt, $(C_5H_{14}ONCl)_2 \cdot PtCl_4$, crystallizes in reddish-yellow plates which are insoluble in alcohol and melt at 240–245° (dec.). Also see (2-hydroxyethyl)trimethyl-C_1^{14}-ammonium chloride, Note 2.

2. Choline chloroplatinate is oxidized with alkaline permanganate according to du Vigneaud.[2] The trimethylamine formed is swept in a current of carbon dioxide into traps containing 0.3 *N* hydrochloric acid. The amine hydrochloride is converted to the chloroplatinate which, since the salt cannot be assayed directly with consistent results, is converted to barium carbonate by wet combustion. The activity of the C^{14}-ethanol moiety is calculated by difference between the C^{14}-choline chloroplatinate and this barium carbonate. Since the carbinol carbon is nonisotopic,[3] the preparative reaction does not involve the intermediary formation of ethylene-C_1^{14} oxide from the 2-chloroethanol-2-C^{14}.

C. Other Preparations

(3-Hydroxypropyl)trimethyl-C_1^{14}-ammonium chloride has been prepared[4] from trimethylamine-C_1^{14} and 3-chloropropanol.

[1]D. D. Van Slyke, J. Folch and J. Plazin, J. Biol. Chem., *136*, 509 (1940).

[2]V. du Vigneaud, M. Cohn, J. P. Chandler, J. R. Schenck and S. Simmonds, J. Biol. Chem., *140*, 625 (1941).

[3]D. B. Sprinson, private communication to Arnstein.

[4]R. M. Lemmon, M. A. Parsons and D. M. Chin, J. Am. Chem. Soc., 77, 4139 (1955).

(2-HYDROXYETHYL-1-C^{14})TRIMETHYLAMMONIUM CHLORIDE (Choline-β-C^{14} Chloride)

$$ClCH_2C^*OOC_2H_5 \xrightarrow{(CH_3)_2NH} \underset{(a)}{(CH_3)_2NCH_2C^*OOC_2H_5} \xrightarrow{LiAlH_4}$$

$$\underset{(b)}{(CH_3)_2NCH_2C^*H_2OH} \xrightarrow{CH_3I} \underset{(c)}{[(CH_3)_3NCH_2C^*H_2OH]^+ I^-} \xrightarrow[HCl]{Ag_2O}$$

$$\underset{(d)}{[(CH_3)_3NCH_2C^*H_2OH]^+ Cl^-}$$

W. G. Dauben and M. Gee, J. Am. Chem. Soc., 74, 1078 (1952).

A. Procedure

(a) N,N-*Dimethylglycine-1-*C^{14} *Ethyl Ester.* The crude ethyl chloroacetate-1-C^{14}, prepared from 7.3 mmoles of sodium acetate-1-C^{14} in 75-90% yield, is allowed to react for 12 hours at room temperature with 1.2 g. (26 mmoles) of dimethylamine in 5 ml. of dry benzene (Note 1). The

precipitated dimethylamine hydrochloride is filtered off and washed with absolute ether. The product is distilled through a microfractionation column (Note 2); yield 55%, b.p. 144–149°.

(b) *2-Dimethylaminoethanol-1-C^{14}.* The *N,N*-dimethylglycine-1-C^{14} ethyl ester is reduced with a solution of 2.0 g. (5.2 mmoles) of lithium aluminum hydride in 25 ml. of anhydrous diethyl carbitol. The mixture is decomposed with 30 ml. of butyl carbitol, then heated at 160–180° for 4 hours. The bath temperature is raised (Note 3), and 25 ml. of distillate is collected in an ice-cooled receiver. The distillate is redistilled up to 180° (Note 4).

(c) *(2-Hydroxyethyl-1-C^{14})trimethylammonium Iodide.* The crude 2-dimethylaminoethanol-1-C^{14} is refluxed with excess methyl iodide on a steam-bath for 2–3 hours. The product is filtered off, washed with absolute alcohol and dried; yield 0.420 g. (Note 5).

(d) *(2-Hydroxyethyl-1-C^{14})trimethylammonium Chloride.* A mixture of 0.752 g. (3.3 mmoles) of the iodide, 1.0 g. (4.3 mmoles) of silver oxide and 5 ml. of water is shaken and stored overnight. The precipitate is centrifuged, and the aqueous layer is separated and titrated to pH 7.0 with *N* hydrochloric acid. The solution of product is lyophilized; yield 0.396 g. (87%). The product may be assayed[1] directly as the chloroplatinate, or the mercuric chloride complex (Note 6).

B. Notes

1. The flask is fitted with a Dry Ice condenser.

2. The distillation separates the aminoester from any amide that may form. Considerably more amide is formed when the aminolysis is performed on the more reactive methyl ester. Several stills for the distillation of centigram and decigram quantities at atmospheric or reduced pressure are described by Gould, Holzman and Niemann.[2] The efficiency is equivalent to approximately 9 theoretical plates, and the column hold-up is 20–30 microliters.

3. Diethyl carbitol, b.p. 188°; butyl carbitol, b.p. 231°; 2-dimethylaminoethanol, b.p. 135°.

4. The presence of small amounts of diethyl carbitol does not interfere with the methylation in the next step.

5. The yield is 15.8% based on sodium acetate-1-C^{14}, from which the starting ethyl chloroacetate-1-C^{14} is prepared.

6. The oxidation of choline to $BaC^{14}O_3$ by wet combustion is unsatisfactory (52%).

[1] H. R. V. Arnstein, J. Chem. Soc., *1952*, 4527.

[2] C. W. Gould, Jr., G. Holzman and C. Niemann, Anal. Chem., *20*, 361 (1948).

(2-ACETOXYETHYL-C_2^{14})TRIMETHYLAMMONIUM BROMIDE
[Acetyl(C_2^{14}-choline) Bromide]

$$C^*H_2{=}C^*H_2 \xrightarrow[H_2O]{CH_3CONHBr} \underset{(a)}{C^*H_2BrC^*H_2OH} \xrightarrow{(CH_3)_3N}$$

$$\underset{(b)}{[(CH_3)_3NC^*H_2C^*H_2OH]^+ Br^-} \xrightarrow{Ac_2O} \underset{(c)}{[(CH_3)_3NC^*H_2C^*H_2OCOCH_3]^+ Br^-}$$

D. E. Walz, M. Fields and J. A. Gibbs, J. Am. Chem. Soc., 73, 2968 (1951).

A. Procedure

(a) *2-Bromoethanol-C_2^{14}*, (*Ethylene-C_2^{14} Bromohydrin*). Ethylene-C_2^{14} (20 mmoles) is vacuum-distilled into a mixture of 2.76 g. (20 mmoles) of *N*-bromoacetamide, 50 ml. of water and 2.0 ml. of 0.1 *N* sulfuric acid contained in a 500-ml. round-bottomed flask equipped with a break-seal. The flask is sealed and shaken at room temperature for 18 hours; then unreacted ethylene-C_2^{14} is recovered by distillation (Note 1). The reaction mixture is saturated with sodium bromide, and the product is extracted repeatedly into ether and dried over sodium sulfate. Distillation yields the product as an almost colorless liquid, 90% yield (Note 2).

(b) (*2-Hydroxyethyl-C_2^{14}*)*triethylammonium Bromide*, (*C_2^{14}-Choline Bromide*). Ethylene-C_2^{14} bromohydrin (1.40 g., 11.2 mmoles) is treated with an excess of anhydrous trimethylamine in 10 ml. of dry ether, and the mixture is stored for 5 hours at 55°, and for 15 hours at room temperature. Distillation of ether and excess amine leaves 1.70 g. (83%) of product, m.p. 275–280° (dec.). Recrystallization from ethanol-ether gives 1.63 g., m.p. 287–289° (dec.) (Note 3).

(c) (*2-Acetoxyethyl-C_2^{14}*)*trimethylammonium Bromide*. (2-Hydroxyethyl-C_2^{14})trimethylammonium bromide (5 mmoles) is refluxed for 4 hours with acetic anhydride under an atmosphere of nitrogen. After separation of the excess anhydride, the yield is 0.97 g. (86%) of slightly colored product, m.p. 143–144° (sintering at 140°). Recrystallization from ethanol-ether gives a pure product melting at 143–144°.

B. Notes

1. Preliminary experiments have shown good agreement between the recovered gas and the unreacted *N*-bromoacetamide, which may be determined by titrating an aliquot of the reaction mixture with standard thiosulfate solution.

2. Addition of hypohalous acid to ethylene yields the halohydrin contaminated with substantial quantities of dihalide. Ethylene bromohydrin has been prepared[1] in 70% yield by passing a mixture of ethylene and bromine vapors into water. An excess of ethylene is required over that

absorbed, and the method does not seem to be readily adaptable to a small-scale synthesis of high specific activity.

3. The product is dissolved in absolute alcohol, to which dry ether is added gradually until precipitation is complete.

C. Other Preparations

(2-Acetoxyethyl)trimethyl-C_1^{14}-ammonium chloride, [acetyl(C_1^{14}-choline) chloride], is prepared[2] in a similar manner by heating the dry C_1^{14}-choline chloride with acetic anhydride at 100° for 2 hours in a sealed tube. The product is crystallized from dry acetone; yield 42%. Lemmon[3] has reported the preparation by refluxing the reactants for 2–3 hours.

[1]J. Read and R. C. Hook, J. Chem. Soc., *1920*, 1214; F. H. McDowall, J. Chem. Soc., *1926*, 499.

[2]F. Christenson and B. M. Tolbert, Nucleonics, 7 (4), 56 (1950).

[3]R. M. Lemmon, M. A. Parsons and D. M. Chin, J. Am. Chem. Soc., 77, 4139 (1955).

(CARBOXYMETHYL)TRIMETHYL-C_1^{14}-AMMONIUM CHLORIDE (C_1^{14}-Betaine Hydrochloride)

$$C^*H_3NH_2 \xrightarrow{BrCH(COOH)_2} C^*H_3NHCH(COOH)_2 \xrightarrow{\Delta}$$

$$C^*H_3NHCH_2COOH \xrightarrow[(HCl)]{\substack{(CH_3)_2SO_4\\KOH}} \left[(CH_3)_2\overset{\displaystyle C^*H_3}{\overset{|}{N}}CH_2COOH\right]^+ Cl^-$$

(a) (b)

E. M. Gal, R. E. Spenger and D. M. Greenberg, J. Org. Chem., *15*, 1261 (1950).

A. Procedure

(a) N-*Methyl-C^{14}-glycine*, (*C_1^{14}-Sarcosine*). Methylamine-C^{14} hydrochloride (2.58 g., 38.2 mmoles) is mixed with 5.62 g. (46.7 mmoles) of sodium methoxide (Note 1) in a 50-ml. round-bottomed flask fitted with a dropping funnel. A solution of 1.3 g. (7.1 mmoles) of bromomalonic acid in 5 ml. of methanol is contained in a 60-ml. glass bomb equipped with a pressure stopcock. Both vessels are frozen with liquid nitrogen and evacuated at a high-vacuum manifold. Methyl alcohol (10 ml.) is added to the flask, and the contents are distilled into the still-frozen bomb (Note 2). The bomb is closed and stored for 3 days in a refrigerator. Crystallization of (methylamino-C^{14})malonic acid is complete by this time. The excess methylamine-C^{14} is recovered by distillation into a flask contain-

ing an excess of frozen *N* hydrochloric acid (Note 3). The residue is washed with two 5-ml. portions of methanol, then the crystals are transferred into a centrifuge tube and dissolved in 20 ml. of water. A solution of 2.68 g. of lead acetate trihydrate in 5 ml. of water is added, and the tube is placed in a refrigerator for 2 days. The crystals of lead (methylamino-C^{14})malonate are separated by centrifuging (Note 4) and suspended in 20 ml. of water. The mixture is saturated with hydrogen sulfide, and the lead sulfide is centrifuged and washed. The combined solutions are evaporated under vacuum at 35–40° to give colorless (methylamino-C^{14})-malonic acid. Decarboxylation of this intermediate is accomplished by slow heating in an oil-bath to 160°, then cooling with protection from moisture. The yield of colorless powder is 0.3506 g. (3.93 mmoles). A second crop of product, obtained by processing material recovered from the mother liquors (Note 4), raises the total yield to 0.4036 g. (76%).

(b) (*Carboxymethyl*)*trimethyl-*C_1^{14}*-ammonium Chloride.* *N*-Methyl-C^{14}-glycine (0.270 g.) is neutralized with potassium hydroxide in a minimum of water, then treated, alternately in small portions (Note 5), with 0.560 g. of potassium hydroxide (in 5 ml. of water) and 1.0 ml. of methyl sulfate. The solution is boiled for 15 minutes, cooled, neutralized with 5% sulfuric acid (2.2 ml.) and concentrated under vacuum at 60° to a syrup, which is extracted with three 15-ml. portions of 95% alcohol. The combined extract is diluted with 45 ml. of water, and the solution is concentrated again to a syrup, which is redissolved in a minimum of hot water. A small portion of *N* hydrochloric acid is added; the solution is refluxed for 30 minutes, and a 20% solution of barium chloride is added until no further precipitate forms. The mixture is centrifuged and separated by filter stick, and the precipitate is thoroughly washed with water. The combined solution is digested on a steam-bath for 3 hours (Note 6), then evaporated to dryness under reduced pressure, and the residue is extracted with two portions of 95% alcohol. The solvent is removed under reduced pressure, and the residue is recrystallized from hot absolute ethanol; a second crop of product is obtained by diluting the filtrate with absolute ether, making the yield 0.375 g. (80%). The molar specific activity is 81.9% of that of the starting material (Note 7).

B. Notes

1. $NaOCH_3 \cdot 2CH_3OH$.

2. The last traces of alcohol and amine are driven over by warming the flask to 35°.

3. Since the methanol bumps, a Kjeldahl trap is used to connect the bomb to the vacuum line. The evaporated distillate yields 1.10 g. of methylamine-C^{14} hydrochloride.

4. The mother liquor is returned to the refrigerator to allow further crystallization.

5. The solution is kept alkaline.

6. Water is added occasionally to prevent going to dryness. The concentrate is tested for sulfate with a drop of barium chloride solution.

7. On the basis of molar specific activity, it is assumed that 18% exchange took place between methyl sulfate and *N*-methyl-C^{14}-glycine. An analytical titration is described by Walker.[1,2]

C. Other Preparations

(Carboxymethyl)trimethyl-C_1^{14}-ammonium chloride has been prepared by Ferger and du Vigneaud,[3] in over 30% yield, by condensing methyl-C^{14} iodide with sodium dimethylaminoacetate, and purifying the betaine through the reineckate.[2]

The preparation by oxidation of (2-hydroxyethyl)trimethyl-C_1^{14}-ammonium chloride with potassium permanganate in faintly acid medium has been reported by Challenger;[4] yield 86%, m.p. 238° (picrate, m.p. 184°). The molar specific activity of each compound was the same.

The preparation of (carboxymethyl)trimethyl-C_1^{14}-ammonium chloride by reacting trimethylamine-C_1^{14} with chloroacetic acid in methanol solution at room temperature has been reported by Lemmon.[2] The product was separated from the excess of starting acid on a column of Dowex-50.

The conversion of C_1^{14}-choline hydrochloride to C_1^{14}-betaine hydrochloride by acidic permanganate oxidation of the free base derived from the mercuric chloride complex has been described by Elwyn;[5] yield 65–80%. The conversion of betaine to trimethylamine (20–25% yield) has been described by Bregoff[6] with the aid of an apparatus diagram.

N-Methyl-C^{14}-glycine hydrochloride has been prepared by Mackenzie,[7] by condensing methyl-C^{14} iodide with *N*-*p*-toluenesulfonylglycine at 70°, hydrolyzing the *N*-methyl-C^{14}-*N*-*p*-toluenesulfonylglycine in concentrated hydrochloric acid with formation of the *N*-methyl-C^{14}-glycine *p*-toluenesulfonic acid salt, m.p. 186–187° (cor.), which was converted to the hydrochloride, m.p. 170–171° (cor.).

[1]H. G. Walker and R. Erlandsen, Anal. Chem., *23*, 1309 (1951).

[2]R. M. Lemmon, M. A. Parsons and D. M. Chin, J. Am. Chem. Soc., *77*, 4139 (1955).

[3]M. F. Ferger and V. du Vigneaud, J. Biol. Chem., *185*, 53 (1950).

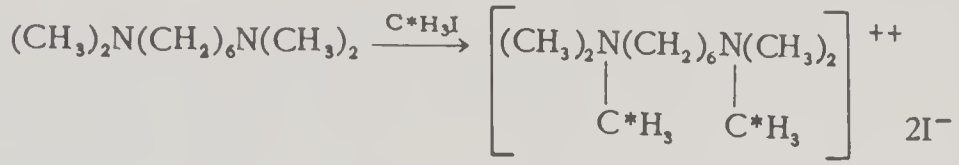
[4]F. Challenger, D. B. Lisle and P. B. Dransfield, J. Chem. Soc., *1954*, 1760; W. Lintzel and S. Fomin, Biochem. Z., *238*, 438 (1931).

[5]D. Elwyn, A. Weissbach, S. S. Henry and D. B. Sprinson, J. Biol. Chem., *213*, 281 (1955).

[6]H. M. Bregoff and C. C. Delwiche, J. Biol. Chem., *217*, 819 (1955).

[7]C. G. Mackenzie, J. Biol. Chem., *186*, 351 (1950); E. Fischer and M. Bergmann, Ann., *398*, 96 (1913).

HEXAMETHYLENEBIS(TRIMETHYL AMMONIUM-C_1^{14} IODIDE) (C_1^{14}-Bistrium)



A. Murray III, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, unpublished work.

A. Procedure

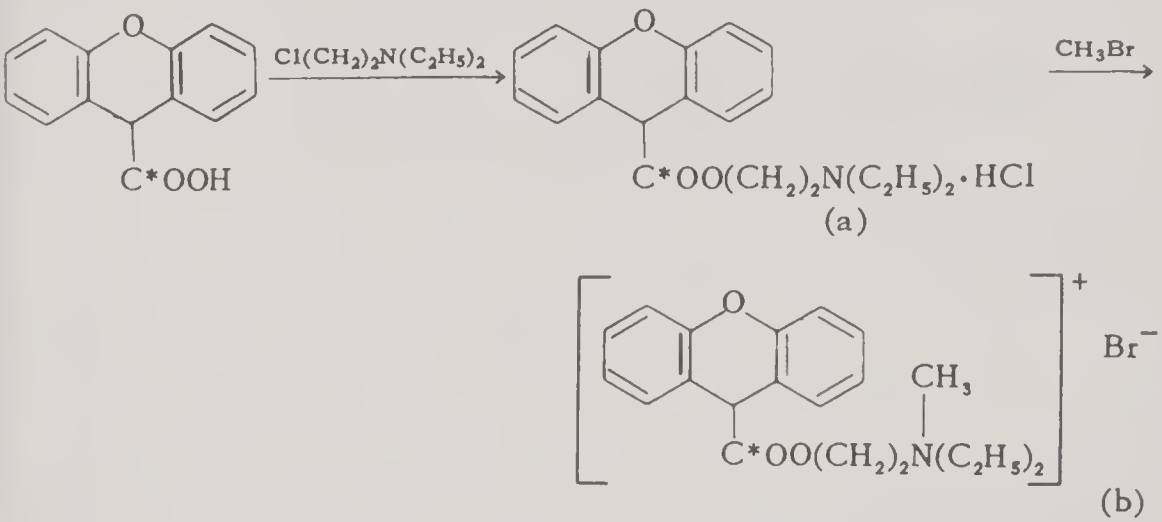
To a frozen mixture of 0.4579 g. (2.66 mmoles) of hexamethylenebis-(dimethylamine) and 3 ml. of dry methanol is added 0.3807 g. (2.68 mmoles) of methyl-C^{14} iodide by vacuum distillation with liquid nitrogen cooling. The flask is closed, and the mixture is stirred for 22 hours at room temperature in the dark. The mixture is frozen, an excess of nonisotopic methyl iodide (0.526 g., 3.71 mmoles) is added by distillation, and the reaction is completed by stirring for 24 hours. The mixture is distilled to dryness under vacuum, and the product is extracted into 5 ml. of aqueous solution which is decolorized with carbon and evaporated. After being dried in a vacuum desiccator, the yield of crude product is 1.1987 g. (98.0%). Recrystallization is accomplished by dissolution in 20 ml. of dry methanol, followed by treatment with 3 volumes of absolute ether; yield 1.1726 g. (95.9%), m.p. 276° (dec.).

B. Other Preparations

In a similar manner is prepared dodecyl-1-C^{14}-trimethylammonium bromide.[1] A mixture of 2.15 g. of 1-bromododecane-1-C^{14} and an excess of 25% alcoholic trimethylamine solution was heated at 120° for 12 hours in a sealed tube. The product was precipitated with ether, and recrystallized from absolute alcohol-ether; yield 54.2%, m.p. 217–219°.

[1]D. Jerchel, H. Becker and K. Schmeiser, Z. Naturforsch., *9b*, 169 (1954).

DIETHYLMETHYL[2-(9-XANTHENECARBONYLOXY-C^{14})ETHYL]-AMMONIUM BROMIDE
(C_1^{14}-Methantheline Bromide)



R. Nakai, M. Sugii and H. Tomono, Bull. Inst. Chem. Res., Kyoto Univ., 33 (5), 211 (1955).

A. Procedure (Note 1)

(a) *2-Diethylaminoethyl 9-Xanthenecarboxylate-C^{14} Hydrochloride.* A mixture of 0.63 g. of 2-chloro-*N,N*-diethylethylamine (Note 2), 1.043 g. of 9-xanthenecarboxylic-C^{14} acid and 6 ml. of isopropyl alcohol is refluxed for 3 hours. The cooled solution is filtered, the filtrate is evaporated on a steambath, and the oil is stored overnight in a desiccator to crystallize. The product is collected and washed with ether and ethyl acetate; yield 1.032 g., m.p. 140–143° (Note 3).

(b) *Diethylmethyl[2-(9-xanthenecarbonyloxy-C^{14})ethyl]ammonium Bromide, (2-Diethylaminoethyl 9-Xanthenecarboxylate-C^{14} Methobromide).* To a solution of 0.856 g. of the ester hydrochloride, 2 ml. of absolute ethanol and 2.76 ml. of 0.862 *N* alcoholic potassium hydroxide, in a 40-ml. heavy-walled, closed flask cooled with an ice-salt-bath, is added 10 ml. of 20% alcoholic methyl bromide solution. The mixture is stored overnight at room temperature; then the potassium chloride is filtered off, and the filtrate is worked up as above; yield 0.888 g. Purification is accomplished by recrystallization from alcohol-ether; yield 0.759 g., m.p. 170–173°. The molar specific activity is 93% of that of the starting acid.

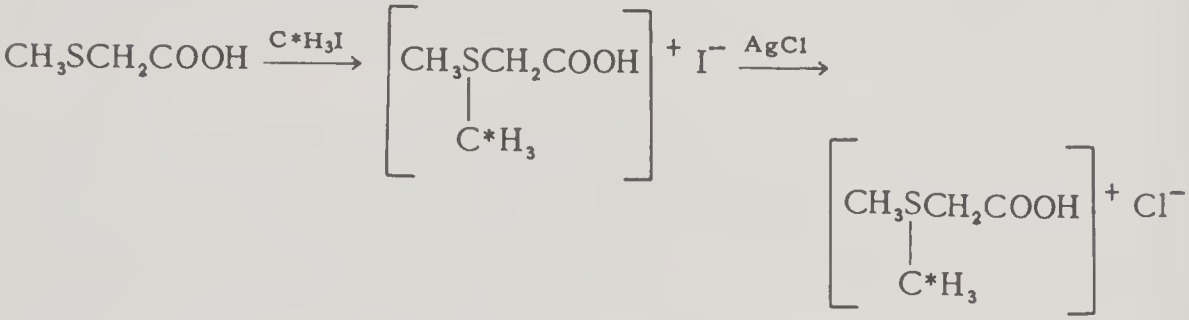
B. Notes

1. The procedure is an adaptation of that of Burtner and Cusic.[1]
2. The starting chloride is prepared by the method of Slotta and Behnisch.[2]

3. The ester hydrochloride melts[1] at 159–160°, after recrystallization from isopropyl alcohol-ethyl acetate.

[1]R. R. Burtner and J. W. Cusic, J. Am. Chem. Soc., *65*, 1582, 262 (1943).
[2]K. H. Slotta and R. Behnisch, Ber., *68*, 754 (1935).

(CARBOXYMETHYL)DIMETHYL-C_1^{14}-SULFONIUM CHLORIDE
(2,2-Dimethyl-C_1^{14}-thetin Hydrochloride)



M. F. Ferger and V. du Vigneaud, J. Biol. Chem., *185*, 53 (1950).

A. Procedure (Note 1)

The methyl-C^{14} iodide, prepared from 4.13 mmoles of methanol-C^{14}, is distilled in a stream of dry nitrogen into a mixture of 0.465 g. (4.37 mmoles) of (methylthio)acetic acid, 3 ml. of formic acid and 0.3 ml. of water in a trap cooled with a Dry Ice-bath. The receiver is stoppered and stored at room temperature for 64 hours. The mixture is dissolved in water and shaken with 0.8 g. of silver chloride, and the silver salts are filtered off and washed with hot water. The combined filtrates are evaporated to dryness, and the residue is recrystallized three times by dissolution in boiling ethanol and precipitation with absolute ether; yield 0.374 g., 58% based on methanol.

B. Notes

1. Thioethers readily add alkyl halides by the method described by Toennies and Kolb,[1] forming sulfonium salts analogous to the halogen derivatives of the basic radicals.

C. Other Preparations

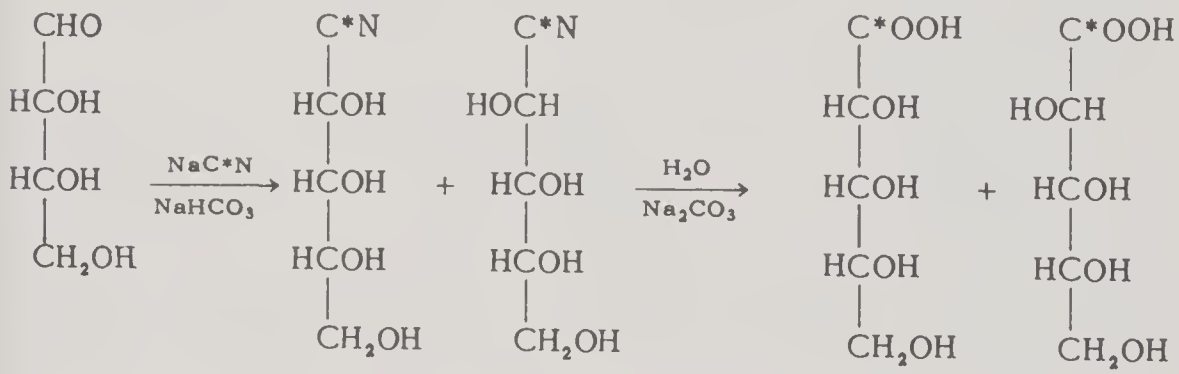
(2-Carboxyethyl)dimethyl-C_1^{14}-sulfonium chloride, (dimethyl-C_1^{14}-β-propiothetin hydrochloride) has been prepared in 73% yield by the same procedure, starting with 3-(methylthio)propionic acid.[2]

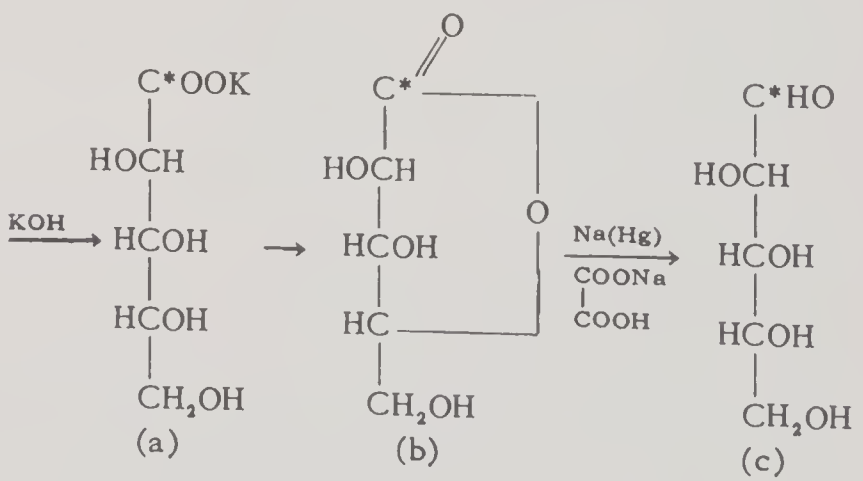
[1]G. Toennies and J. J. Kolb, J. Am. Chem. Soc., *67*, 849 (1945).
[2]A. J. Haagen-Smit, J. G. Kirchner, C. L. Deasy and A. N. Prater, J. Am. Chem., Soc., *67*, 1651 (1945); B. Holmberg, Arkiv Kemi, Mineral. Geol., *B 21* (7), 7 (1945); through Chem. Abstracts, *41*, 4465 (1947).

SUGARS AND SUGAR DERIVATIVES

ARABINOSE-1-C^{14}

METHOD I





H. L. Frush and H. S. Isbell, J. Research Nat. Bur. Standards, *51*, 307 (1953).

A. Procedure

(a) *Potassium D-Arabonate-1-*C^{14} (Note 1). To a tube containing a solution of 3.1 mmoles of sodium cyanide-C^{14}, 5 mmoles of sodium hydroxide and 10 ml. of water, frozen on the sides by cooling in a Dry Ice-bath, is

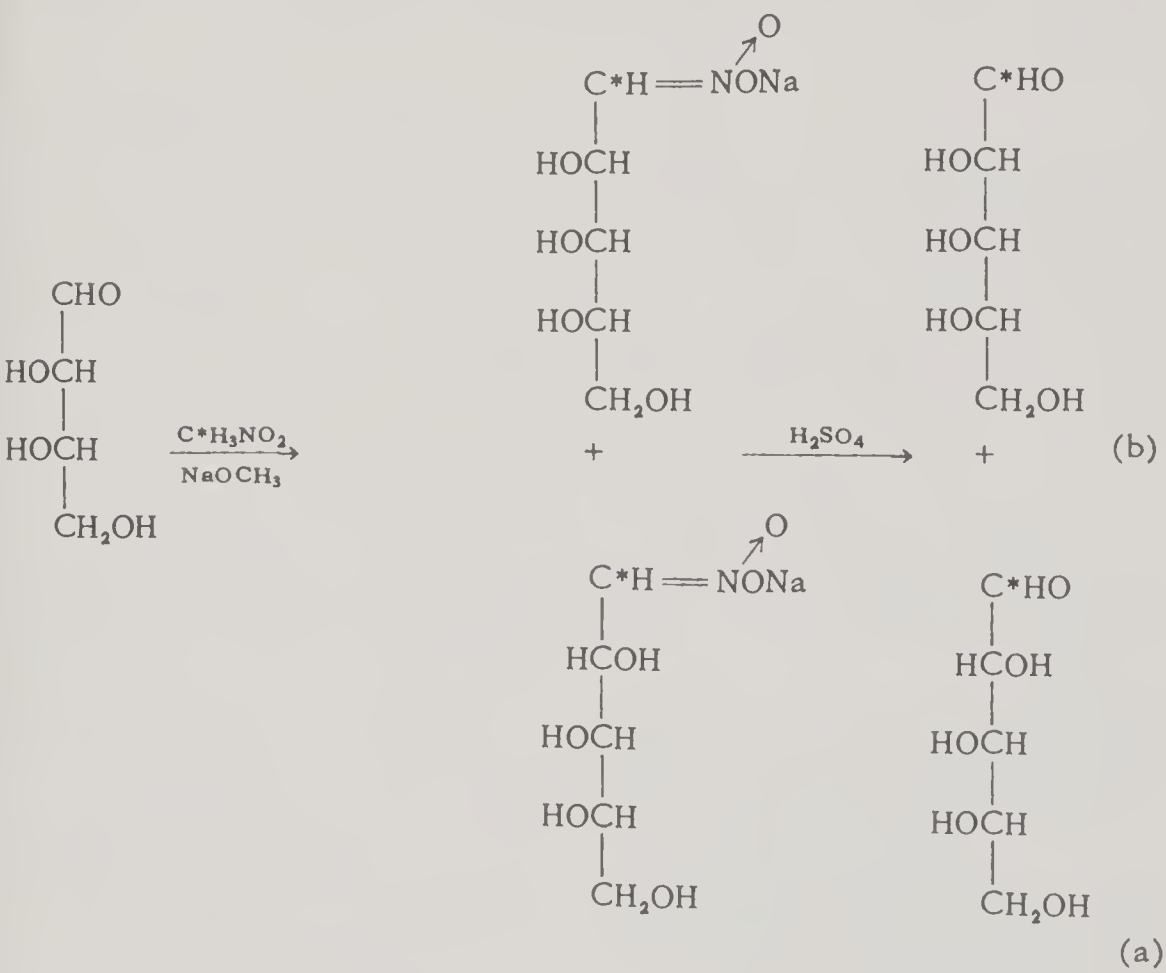
added a small lump of carbon dioxide and a solution of 5 mmoles of sodium bicarbonate and 4 mmoles of D-erythrose in 10 ml. of water (Note 2). The mixture is thawed by shaking the loosely stoppered tube in an ice-bath, then stored at 0° for 1 day and at room temperature for 5 days. The nitriles are hydrolyzed by heating the mixture on a steam-bath for 7 hours in a stream of air, with addition of water as required. The residue is dissolved in water and passed through a cation exchange column (Note 3) with thorough washing. The combined effluent and washings are concentrated under reduced pressure to about 25 ml. and neutralized with potassium hydroxide. The mixture is diluted with 2.0 g. of carrier, concentrated to 4 ml., diluted with methanol and stored in a refrigerator to crystallize. The product is collected, recrystallized from water-ethanol, washed with cold, aqueous ethanol (1:4) and dried in a vacuum desiccator; yield 2.23 g., 42.7% on a radiochemical basis. The yield may be raised to 50% by recrystallizing 2.5 g. of carrier, in 3 portions, from the mother liquor (Note 4).

(b) *D-Arabono-γ-lactone-1-C*^{14}. An aqueous solution containing 0.446 g. (2.19 mmoles) of potassium D-arabonate-1-C^{14} is passed through a cation exchange column of Amberlite IR-120 with thorough washing. The effluent and washings are evaporated to a syrup under reduced pressure, and the D-arabonic-1-C^{14} acid is transferred with a few drops of water to a reduction tube (see D-mannose-1-C^{14}). The solution is diluted with 5 ml. of glacial acetic acid and heated for 5 hours in a boiling water-bath, under an air stream. At 1 hour the mixture is treated with 1 ml. of water and 5 ml. of glacial acetic acid, followed by three 5-ml. portions of acetic acid at 1-hour intervals. The pale yellow residue is dissolved in a few drops of methanol, seeded and stored over calcium chloride for 1 day (Note 5). The yield, based upon optical rotation data, is 91 and 94% after 24 and 72 hours, respectively.

(c) *D-Arabinose-1-C*^{14}. To an ice-cooled, vigorously stirred mixture of 6.4 g. of crystalline sodium acid oxalate, 20 ml. of water and the D-arabono-γ-lactone-1-C^{14} is added 9.2 g. of 5% sodium amalgam pellets (Note 6). After 3 hours of stirring, the mercury is removed, the solution is diluted with 5 volumes of methanol, and the precipitated salts are filtered off and washed with methanol. The filtrate is cooled, made just basic to phenolphthalein with aqueous sodium hydroxide, concentrated under reduced pressure to 10 ml. and diluted with 5 volumes of methanol, and the precipitated salts are filtered off and washed with methanol. The alcohol is evaporated under diminished pressure, and the aqueous solution is passed through a deionizing column (Note 7) with thorough washing. The combined solution is concentrated to 10 ml. under reduced pressure (below 40°) and filtered through a bed of decolorizing charcoal

(Note 8) into a 50-ml. flask, where the filtrate and washings are lyophilized. The residue is dissolved in several drops of methanol and treated with isopropyl alcohol to incipient turbidity, then seeded and stored for several days to crystallize. The mother liquor is removed, and the product is washed with a few drops of methanol - 2-propanol (2 : 1) and recrystallized from methanol solution by treatment with isopropyl alcohol; yield 0.185 g., 56.7% on a radiochemical basis. The yield may be raised to 60% by treating the mother liquor with 0.100 g. of carrier (Note 9).

METHOD II



D. A. Rappoport and W. Z. Hassid, J. Am. Chem. Soc., 73, 5524 (1951).

A. Procedure (Note 10)

Sodium 1-Deoxy-1-nitroarabitol-1-C^{14} *and Sodium 1-Deoxy-1-nitroribitol-1-C*^{14}. Into a mixture of 3.60 g. (30 mmoles) of L-erythrose (Note 11) and 10 ml. of absolute methanol, added to a cooled solution of 0.368 g. (16 mmoles) of sodium in 10 ml. of methanol, is vacuum-distilled 6.1 g. (10

mmoles) of nitromethane-C^{14} (Note 12), with Dry Ice-cooling. The mixture is stored at room temperature for 20 hours, then cooled to 0° and diluted with an equal volume of ether. The precipitated sodium salts are collected by filtration, washed with methanol-ether (1 : 1), ether, and petroleum ether, and dried under vacuum; yield 3.2 g., 77% based on the nitromethane-C^{14} used.

"*Pentose-1-C*^{14}". To 9.0 ml. of 18 *N* sulfuric acid at −10 to −15° is added dropwise, with stirring, a solution of the sodium nitroalcohols-1-C^{14} in a minimal volume of ice water. The mixture is stirred for 15 minutes, then diluted with 100 ml. of ice water and passed through ion exchange columns of Amberlite IR-100 and Duolite A-4, by washing with water until the effluent activity approaches background. The combined solution is evaporated under reduced pressure; yield 2.2 g. of syrup (Note 13).

(a) *L-Arabinose-1-C*^{14}. The mixture of L-ribose-1-C^{14} and L-arabinose-1-C^{14} is separated by partition chromatography as described by Hough.[1] A concentrated aqueous solution of the pentose mixture is placed on a powdered cellulose column (Note 5) and developed with butanol saturated with water containing 0.3% concentrated ammonia. The location of each constituent is determined in every tenth 1.2-ml. fraction by paper chromatography, using a spray of aniline hydrogen phthalate as a pentose reagent.[2] The L-arabinose-1-C^{14} fractions are combined, evaporated and purified by band chromatography on paper, as follows. The crude L-arabinose-1-C^{14} (0.029 g.) is dissolved in 0.6 ml. of water, and a 0.25-ml. aliquot is deposited in 0.01-ml. portions along a line on two 18 × 22-inch sheets of Whatman No. 1 paper (Note 15). The sheets are chromatographed for 24 hours with phenol saturated with water, then dried and radioautographed. The markers are cut from the paper and sprayed to identify the product band, which then is cut from the paper and eluted with water. The solution is evaporated to dryness under vacuum at 40°, and the residue, dissolved in 0.3 ml. of water, is chromatographed on paper using butanol-ethanol-water (21 : 13 : 5). Elution and evaporation yield 1.2 mg. of very pure product. The total yield is 0.0222 g., over-all radiochemical yield 3% (Note 16).

(b) *L-Ribose-1-C*^{14}. The crude product may be purified by paper chromatography in the manner described above.

B. Notes

1. The procedure is based upon a tracer study of the effect of several catalysts upon the cyanohydrin synthesis, employing the technique of isotopic dilution[3] to determine the yield of a specific epimer. Ammonium

carbonate and sodium bicarbonate favor the formation of the D-arabonic isomer (55.6 and 49.6% yield), while the more basic sodium carbonate reduces the yield to 37.1%.

A similar preparation of the isotopic isomers (90.2% yield) in the arabonate : ribonate ratio of 1.89 has been reported in detail by Neish,[4] the former being isolated as calcium D-arabonate-1-C^{14} and the latter as the usual cadmium salt.

2. A syrup of 90% purity is employed. Directions are given by Frush and Isbell for preparing the starting material by degradation of 4,6-*O*-ethylidene-D-glucose by periodate oxidation. Other preparative methods have been surveyed by Overend;[5] also see Note 11. Directions are given by Neish[4,9] for preparing a chromatographically pure solution of the tetrose by acid hydrolysis of the 2,4-*O*-ethylidine-D-erythrose formed by oxidation of 4,6-*O*-ethylidine-D-glucitol with periodic acid.

3. Amberlite IR-120, 10 ml.

4. The residues are saved for the preparation of cadmium D-ribonate-1-C^{14}.

5. In the absence of heating with acetic acid, the lactonization requires 1–2 weeks.

6. The optimum yield of product (85%) is obtained with 4.6 g. of the amalgam per mmole of lactone. See D-mannose-1-C^{14}, Note 13, for preparation of the reagent.

7. Amberlite IR-100 and Duolite A-4, 12.5 ml. each.

8. This step removes any trace of oil introduced with the amalgam.

9. Radiochemical yields of up to 70% usually are obtained. This compound may be used as an intermediate in the preparation of D-glucose-2-C^{14} (see the method of D-glucose-1-C^{14}).

10. This preparation is based on the method of Sowden[6] and of Fischer, see D-glucose-1-C^{14}, Method II.

11. Directions are given by Rappoport for preparing L-erythrose in good yield by degradation of L-glucose; also see Note 2.

12. The maximum yield is realized with this ratio of reactants.

13. The yield[7] is 30%, when the Nef reaction is conducted at 25–30° at the usual laboratory scale.

14. The column, 22.5 × 0.75 inches, is packed with Whatman No. 1 ashless pellets and attached to a "Technicon" fractionator which collects 400 1.2-ml. fractions of eluate.

15. Nonisotopic L-arabinose is placed along the edge as a marker.

16. Crystalline product might be obtained by dilution of the syrup with carrier. A large-scale preliminary experiment with D-erythrose and nitromethane gave a product which formed D-arabinose phenylosazone, m.p. 160°.

C. Other Preparations

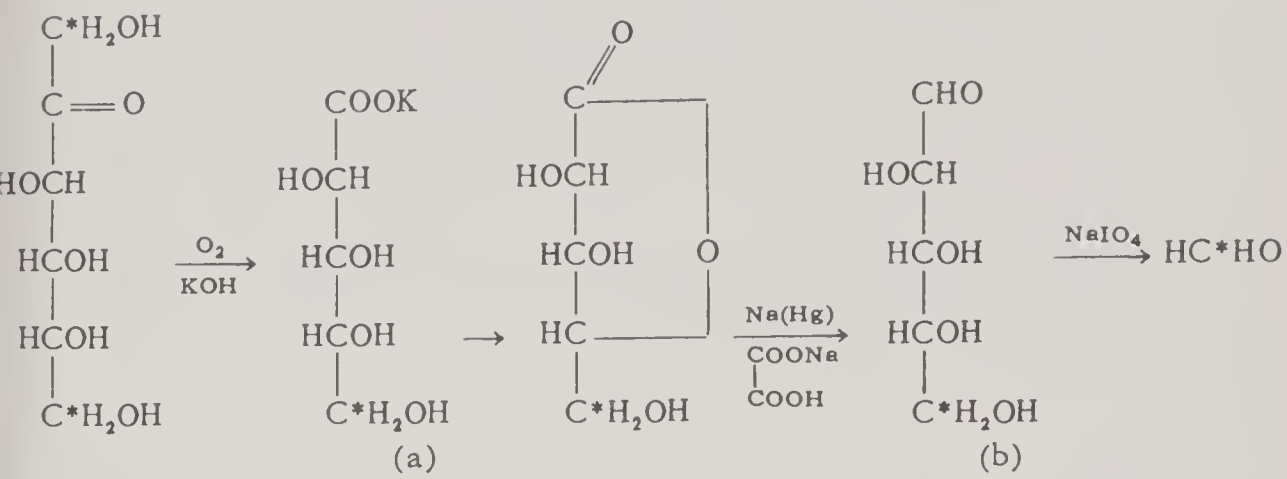
The preparation of D-arabinose-1-C^{14} and D-ribose-1-C^{14} at the 0.3-mmole level, by essentially Method I, has been described in detail by Laland;[8] total over-all yield 25%. The mixed aldonic acids were lactonized and reduced, and the epimeric sugars were separated in the pure state by paper chromatography at the last step. Attempts to induce epimerization of D-arabonic-1-C^{14} acid by heating with pyridine were successful only to the extent of 20%.

Two preparations similar to Method I have been described by Neish.[4,9] D-Erythrose was reacted with potassium cyanide-C^{14} in the presence of equimolar amounts of sodium hydroxide and sodium bicarbonate; 3 or 4 parts of carrier were used to isolate the two products: cadmium D-ribonate-1-C^{14} (35% yield) and calcium D-arabonate-1-C^{14} (53% yield). A higher yield of the ribonic epimer might have been achieved if more alkali had been added during the condensation. The syrupy crude D-ribonic-1-C^{14} acid was lactonized by heating at 90° for 24 hours under reduced pressure, no attempt being made to obtain a crystalline product. The syrupy D-ribono-γ-lactone-1-C^{14} was reduced with sodium amalgam, giving D-ribose-1-C^{14}; yield 44% as determined by hypoiodite oxidation of an aliquot of a solution deionized by use of Amberlite IR-120 and IR-4B (less than 10% loss of the ribose).

The preparation of calcium D-arabonate-1-C^{14} by essentially Method I has been described by David.[10] The reaction mixture was deionized by passage through a column of Zeo-Karb, and titrated with calcium hydroxide (98.3% yield of isomeric acids); yield of crystalline product 44.5%.

[1]L. Hough, J. K. N. Jones and W. H. Wadman, J. Chem. Soc., *1949*, 2511.
[2]S. M. Partridge, Nature, *164*, 443 (1949).
[3]R. H. Mayor and C. J. Collins, J. Am. Chem. Soc., *73*, 471 (1951).
[4]A. C. Neish, Can. J. Chem., *32*, 334 (1954).
[5]W. G. Overend, M. Stacey and L. F. Wiggins, J. Chem. Soc., *1949*, 1358.
[6]J. C. Sowden, J. Am. Chem. Soc., *72*, 808 (1950).
[7]J. U. Nef, Ann., *280*, 263 (1894).
[8]S. G. Laland and I. Smith-Kielland, Acta Chem. Scand., *9*, 393 (1955).
[9]A. C. Neish and A. C. Blackwood, Can. J. Biochem. Physiol., *32*, 323 (1955); A. C. Neish and F. J. Simpson, Can. J. Biochem. Physiol., *32*, 147 (1954).
[10]S. David, Bull. soc. chim. France, (5) *21*, 701 (1954); S. David and J. Renault, *ibid.*, (5) *21*, 61 (1954).

D-ARABINOSE-5-C^{14}



H. L. Frush and H. S. Isbell, J. Research Nat. Bur. Standards, *51*, 167 (1953); Atomic Energy Commission Report, NBS-2308; Nuc. Sci. Abstracts, 7, 2767 (1953).

A. Procedure

(a) *Potassium D-Arabonate-5-C^{14}* (Note 1). A solution of 0.075 g. of D-fructose-1,6-$C^{14}_{1/2}$ in 2 ml. of water is frozen, by Dry Ice-bath cooling, on a portion of the wall of a 200-ml. pressure flask, containing 2 ml. of 2 *N* potassium hydroxide frozen on another portion (Note 2). The flask is evacuated, filled with oxygen at 10 p.s.i. and mechanically shaken at room temperature for 24 hours. The mixture is diluted with 50 ml. of methanol, seeded and stored for 1 day. The crystals are separated by decantation, washed with methanol and recrystallized from water-methanol (Note 3). The molar specific activity is 50.4% of that of the starting material (Note 4). Degradative studies indicate that there is no isotopic rearrangement; see below.

(b) *D-Arabinose-5-C^{14}*. A solution of 0.204 g. (1 mmole) of potassium D-arabonate-5-C^{14} in 10 ml. of water is passed through a cation exchange column (Note 5), followed by 20 ml. of wash water. The effluent and washings are evaporated under reduced pressure, and the residual syrup, D-arabonic-5-C^{14} acid, is lactonized in a reduction tube (Note 6) by heating for several days at 50° in a current of air, with occasional addition of methanol. To the completely crystallized D-arabono-γ-lactone-5-C^{14} is added, after about 1 week, 3.2 g. of crystalline sodium acid oxalate and 10 ml. of water. To the vigorously stirred mixture, with cooling in an ice-bath, is added 4.6 g. of 5 per cent sodium amalgam pellets (Note 7). The mercury from the spent amalgam is removed, the solution is di-

luted with five volumes of methanol, and the precipitated salts are filtered off and washed with methanol. The combined filtrate is concentrated to 5 ml. and again treated with methanol to remove salts. The filtrate is evaporated, and the residue is titrated, with cooling, to the end point of phenolphthalein with sodium hydroxide solution. The filtered solution is passed through a deionizing column of equal parts of Amberlite IR-100 and Duolite A-4. The combined effluent and washings are concentrated under reduced pressure to about 100 ml., then lyophilized. The product is crystallized from 0.5 ml. of methanol by the addition of isopropyl alcohol; yield 0.103 g. (68%), without the use of carrier (Note 7). By use of carrier, the radiochemical yield is 75% based on the potassium D-arabonate-5-C^{14} used.

B. Notes

1. The procedure is an adaptation of the method described by Isbell.[1] The method is useful for the semimicro degradative assay of C-1 of any simple aldose or 2- ketose by oxidation to the next lower aldonic acid. The loss in activity of D-glucose-1-C^{14} and D-fructose-1,6-$C^{14}_{1/2}$ is 100% and 49.6%, respectively, upon removal of C-1.

2. The effects of alkali upon the sugar are deleterious in the absence of oxygen.

3. Constant specific activity is attained in two or three recrystallizations.

4. Directions are given by Frush and Isbell for preparing D-fructose-1,6-$C^{14}_{1/2}$ from D-mannitol-1-C^{14} by microbiological oxidation. The yield of arabonate is 52.4%, based upon the mannitol reacted and without isolation of the fructose.

5. Amberlite IR-120, 10 ml.

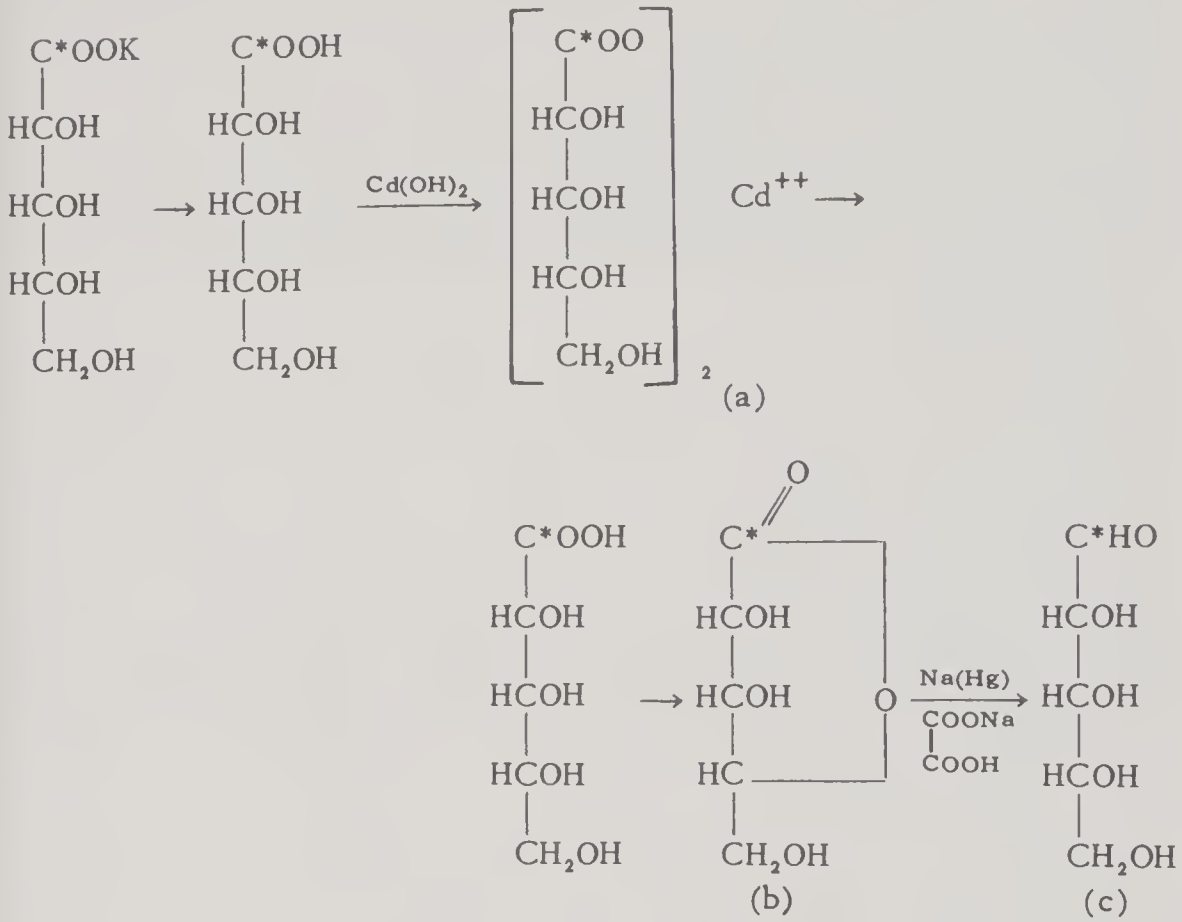
6. See D-mannose-1-C^{14}.

7. Directions are given by Frush and Isbell for degrading the product to formaldehyde-C^{14} (dimedon) by periodate oxidation. The method[2] is general for the quantitative determination of primary carbinol groups.

[1]H. S. Isbell, J. Research Nat. Bur. Standards, *29*, 227 (1942).

[2]R. E. Reeves, J. Am. Chem. Soc., *63*, 1476 (1941).

D-RIBOSE-1-C^{14}



H. L. Frush and H. S. Isbell, J. Research Nat. Bur. Standards, *51*, 307 (1953).

A. Procedure

(a) *Cadmium D-Ribonate-1-C^{14}* (Note 1). The syrupy residue from the preparation of potassium D-arabonate-1-C^{14} is passed through a cation exchange column (Note 2) with thorough washing. The combined effluent and washings are made just basic to phenolphthalein with cadmium hydroxide and diluted with 1.0 g. of carrier; then the solution is filtered through a bed of decolorizing carbon. The filtrate is evaporated under reduced pressure, and the syrup is seeded and treated with aqueous methanol to produce a voluminous, crystalline product. The salt is filtered off and recrystallized from 4 ml. of hot aqueous solution by adding 5 ml. of methanol-water (1 : 1), 2 ml. of methanol-water (2 : 1) and methanol to incipient turbidity, followed by slow cooling. The first crop yield is 0.679 g., 12.8% on a radiochemical basis from sodium cyanide-C^{14}. The yield is raised to 23% by crystallizing two 1.0-g. portions of carrier from the mother liquor.

(b) *D-Ribono-γ-lactone-1-C^{14}*. An aqueous solution containing 0.679 g. of cadmium D-ribonate-1-C^{14} is passed through a cation exchange col-

umn (Note 2) with thorough washing. The combined effluent and washings are concentrated under reduced pressure, then transferred to three reduction tubes (see D-mannose-1-C^{14}) with a little water and evaporated to dryness on a steam-bath with an air stream. Each residue is dissolved in several drops of water, treated with 5 ml. of methyl cellosolve and evaporated; the procedure is repeated four times in 2 days. Each residue is then dissolved in 2 ml. of methyl cellosolve, seeded with the lactone and stored over calcium chloride with occasional moistening of the residue with solvent (Note 3).

(c) *D-Ribose-1-C^{14}*. To each of the samples of D-ribono-γ-lactone-1-C^{14}, mixed with 20 ml. of water and excess sodium acid oxalate, is added 9.2 g. of 5 per cent sodium amalgam per mmole of lactone with vigorous stirring and ice-bath cooling (Note 4). When the amalgam is spent, stirring is stopped and the mercury is separated. The three solutions are combined and diluted with five volumes of methanol; then the precipitated salts are filtered off and washed with methanol. The filtrates are concentrated to 15 ml. under reduced pressure and again treated with methanol for removal of salts. The filtrate is concentrated to 10 ml., to remove methanol, then made just basic to phenolphthalein with aqueous sodium hydroxide and passed through a deionizing column (Note 5). The combined effluent and washings are concentrated under reduced pressure to 6 ml. and mixed with 0.750 g. of carrier. The solution is filtered through a bed of diatomaceous earth and decolorizing carbon, then lyophilized. The syrup is dissolved in 0.5 ml. of alcohol, treated with ethanol-isoamyl alcohol to the point of incipient turbidity, seeded, and stored for about 5 days in a refrigerator. The mother liquor is separated by capillary pipette, and the product is washed with ethanol and dried over calcium chloride. The yield is 0.768 g., 37% on a radiochemical basis from the cadmium salt. The crude product is dissolved in a few drops of water and mixed with one drop of acetic acid; then the solution is filtered through decolorizing carbon and evaporated under vacuum. The syrup is dissolved in ethanol, evaporated, and crystallized in the manner already described. The yield of purified product is 0.540 g.; nearly all of the activity of the crude product may be recovered by reworking the mother liquor with carrier.

B. Notes

1. The synthesis is based upon the production of D-ribonic-1-C^{14} acid as an epimeric by-product in the synthesis of D-arabinose-1-C^{14}.

2. Amberlite IR-120.

3. Crystallization is slow, but all evidence of syrup eventually disappears.

4. Experiment has shown that these conditions afford the maximum yield of product (70%).

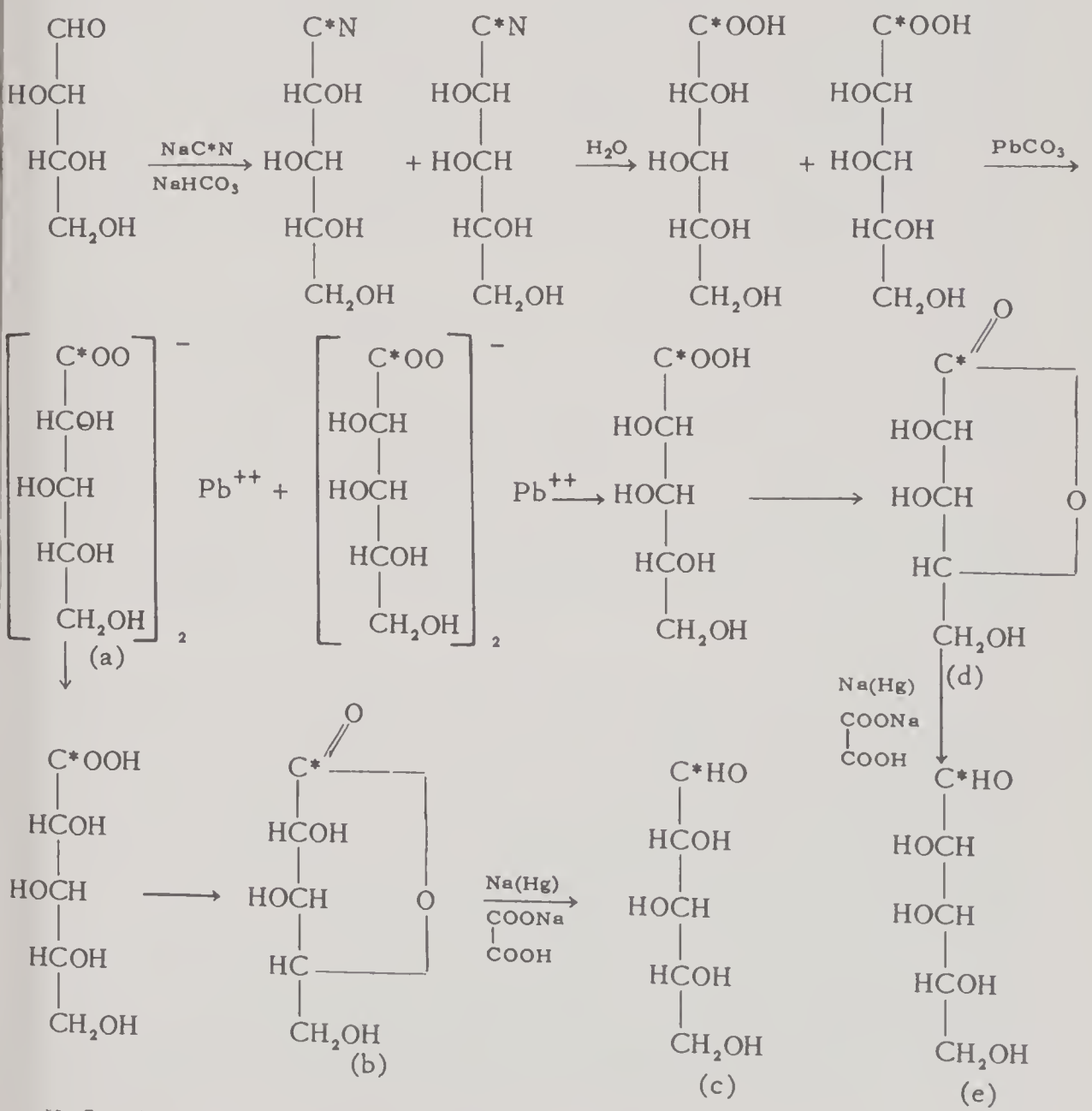
5. Amberlite IR-100 and Duolite A-4, in equal amounts.

C. Other Preparations

The preparation of D-ribose-1-C^{14} by essentially this method has been described by Neish and Laland (see D-arabinose-1-C^{14}).

α-D-XYLOSE-1-C^{14}

METHOD I



H. S. Isbell, H. L. Frush and N. B. Holt, J. Research Nat. Bur. Standards, 53, 325 (1954).

A. Procedure

(a) *Lead D-Xylonate-1-C^{14} Monohydrate.* To a solution of 3.3 mmoles of D-threose (Note 1) and 4.4 mmoles of sodium bicarbonate at 0° is added a solution containing 2.2 mmoles of sodium cyanide-C^{14} and 2.2 mmoles of sodium hydroxide in 10 ml. of water. The mixture is stored at 0° for 1 day and at room temperature for 4 days, then is heated at 80° under an air stream, with occasional additions of water, until ammonia evolution ceases (Note 2). The salt solution is diluted and passed through a column of cation exchange resin by washing thoroughly with water (Note 3). The total effluent is treated with 0.295 g. of lead carbonate, then concentrated to 100 ml. under reduced pressure and filtered. After addition of 0.500 g. of carrier lead D-xylonate monohydrate, the solution is concentrated to 5 ml., treated dropwise with methanol to the point of incipient turbidity and stored for 1 day to crystallize. The product is collected and recrystallized from water by the addition of methanol. The combined mother liquors are concentrated under reduced pressure and worked up with four 0.500-g. portions of carrier. The five combined crops are recrystallized from water-methanol; yield 2.557 g., 34.2% on a radiochemical basis (Note 4).

(b) *D-Xylono-γ-lactone-1-C^{14}.* An aqueous solution of the lead D-xylonate-1-C^{14} is passed through a column containing 20 ml. of Amberlite IR-120H, by washing with 1 l. of water. The effluent is concentrated under reduced pressure; then equal aliquots are transferred to five reduction tubes (Note 5) and evaporated to dryness at 60° in a stream of air. The D-xylonic-1-C^{14} acid is dissolved in a few drops of ethylene glycol monomethyl ether, seeded with the lactone and stored in a desiccator over calcium chloride at room temperature for 2 weeks, the tubes being moistened daily with a few drops of the methyl cellosolve. The yield is quantitative.

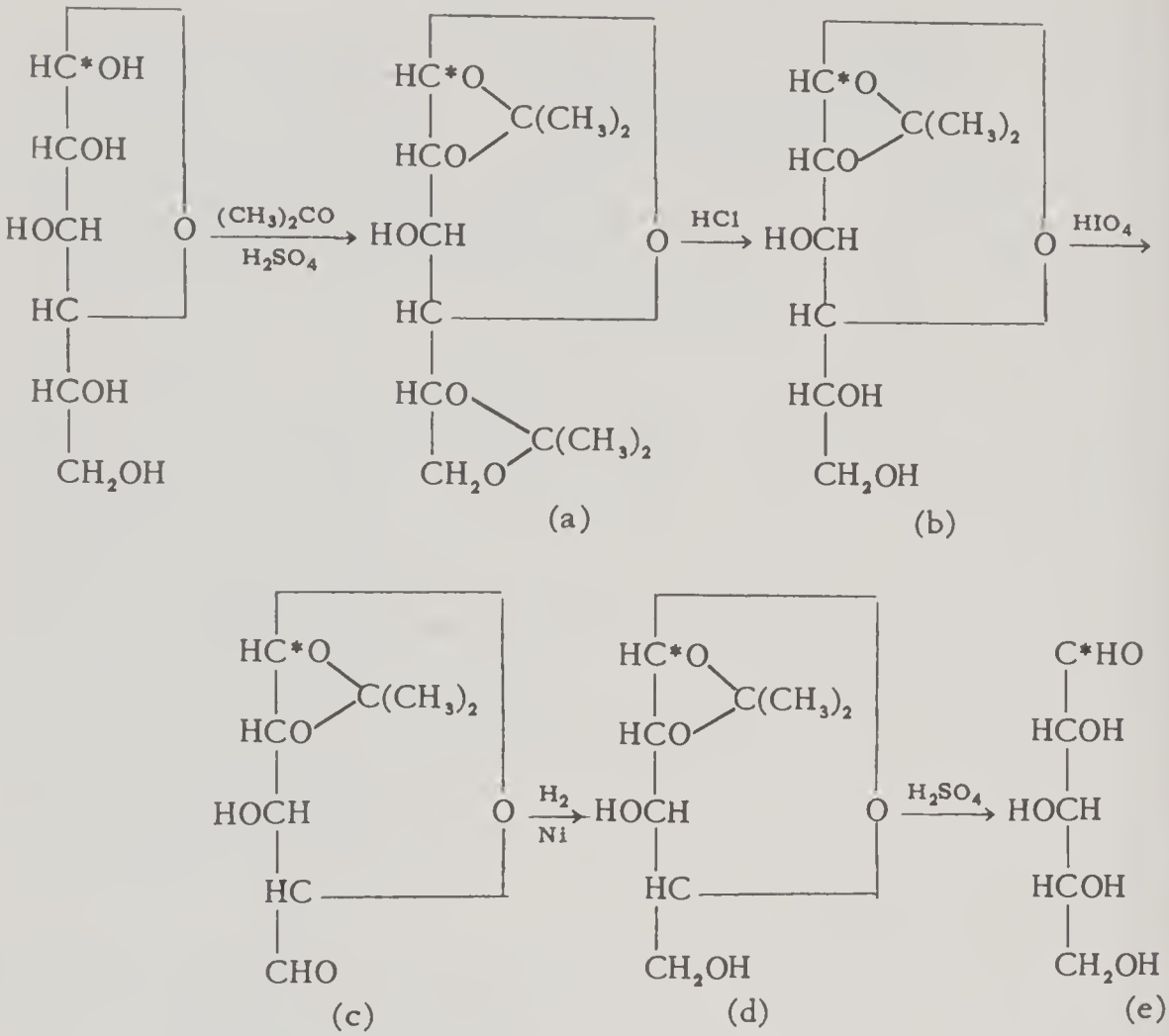
(c) *α-D-Xylose-1-C^{14}.* To an ice-cooled, vigorously stirred solution of the crystalline lactone (1.84 mmoles in each tube) in 20 ml. of ice water containing 5.8 g. of sodium acid oxalate is added 8.3 g. of 5% sodium amalgam pellets (Note 6). When the amalgam is spent, the five mixtures are combined and separated from mercury, and the sodium salts are removed by filtration and washed with ice water. The filtrate is concentrated to 25 ml. under reduced pressure, then cooled to 0°, is made just basic to phenolphthalein with sodium hydroxide and diluted with two volumes of methanol. The precipitated salts are removed by filtration and washed with methanol. An additional crop is obtained by concentrating the filtrate to 10 ml., mixing with 50 ml. of methanol, concentrating to a syrup, then mixing the residue with 25 ml. of methanol and

separating the insoluble material, which is washed with methanol. The filtrate is concentrated under reduced pressure, diluted with water and deionized by passage through a 20-ml. column of mixed cation and anion exchange resins (Note 7). The effluent is concentrated to 25 ml. under reduced pressure, filtered, and lyophilized. The residue is dissolved in 2 ml. of methanol and stored for 24 hours. The product is collected and recrystallized from a few drops of water by dilution with methanol; yield 0.485 g. The radiochemical yield, 34.8% based on lead D-xylonate-1-C^{14}, is raised to 56% by recrystallization of four portions of carrier (total 1.1 g.) from the mother liquor (Note 8).

(d) *D-Lyxono-γ-lactone-1-*C^{14}. The residual liquors containing lead D-lyxonate-1-C^{14}, from the preparation of lead D-xylonate-1-C^{14} monohydrate, are passed through a 10-ml. column of Amberlite IR-120H. The effluent is mixed with 1.0 g. of carrier, and the solution is concentrated to a syrup under reduced pressure. The residue is heated at 90° for 8 hours in a stream of air, with the addition of 0.2-ml. portions of glacial acetic acid at 2-hour intervals. A solution of the residue in 1 ml. of ethanol is treated with 2-propanol to the point of incipient turbidity, then seeded and stored at room temperature for 24 hours. The product is collected and recrystallized from ethanol - 2-propanol; yield 0.574 g. The radiochemical yield, 29.9% based on sodium cyanide-C^{14}, is raised to 48.7% by recrystallization of 0.6 g. of carrier (in three portions) from the combined mother liquors.

(e) *β-D-Lyxose-1-*C^{14}. D-Lyxono-γ-lactone-1-C^{14} (1 mmole) is reduced with the use of 22 g. of 4.2% sodium amalgam, 8 g. of sodium acid oxalate and 20 ml. of water at 0° for 3 hours (Note 9); yield 0.032 g. The radiochemical yield, 21.3% based on the lactone, is raised to 32% by the use of 0.6 g. of carrier in three portions. Hydrolysis of the mother liquors, deionization, concentration and the use of 0.4 g. of carrier (in two portions) raise the radiochemical yield to 64%.

METHOD II



J. C. Sowden, J. Am. Chem. Soc., 73, 5496 (1951).

A. Procedure (Note 10)

(a) *1,2:5,6-Di-O-isopropylidene-D-glucofuranose-1-C*^{14} (Notes 11,12). To a stirred mixture of 5 g. of powdered D-glucose-1-C^{14} and 100 ml. of acetone at 0° is added slowly 4 ml. of sulfuric acid. The mixture is stirred (closed) at room temperature for 4 hours, then filtered from 0.140 g. of unreacted starting material. The solution is made just alkaline, with cooling, by the addition of saturated sodium hydroxide solution. The salt cake is filtered off and washed with acetone. The combined solutions are concentrated under reduced pressure, water is added and the concentration is repeated to a volume of 50–75 ml.

(b) *1,2-O-Isopropylidene-D-glucofuranose-1-C*^{14} (Note 11). The solution of *1,2*:5,6-di-*O*-isopropylidene-D-glucofuranose-1-C^{14} is adjusted to pH 2.0 with concentrated hydrochloric acid and stirred for 4 hours at 40°. The

pH is adjusted to 8–8.5 with sodium hydroxide, and the solution is extracted with an equal volume of benzene to remove unhydrolyzed starting material.

(c) *5-Aldo-1,2-O-isopropylidene-D-xylofuranose-1-C*^{14}. To the stirred, aqueous solution of 1,2-isopropylidene-D-glucose-1-C^{14} is added an excess of solid sodium metaperiodate (Note 13) in small portions, while the pH is maintained near that of the methyl red end point by the dropwise addition of sodium hydroxide solution. The excess oxidant is destroyed with a few drops of glycerol, and the solution is concentrated under reduced pressure. Absolute alcohol is added and the concentration is repeated. The syrupy residue is mixed with sodium sulfate and extracted with five 40-ml. portions of chloroform. Evaporation under reduced pressure gives 5 g. of crude product (Note 14).

(d) *1,2-O-Isopropylidene-D-xylofuranose-1-C*^{14}. The crude 5-aldo-1,2-*O*-isopropylidene-D-xylofuranose-1-C^{14} is mixed with 65 ml. of 95% ethanol and hydrogenated over 5 g. of Raney nickel for 90 hours at room temperature and atmospheric pressure (Note 15). The solution is filtered and concentrated under reduced pressure (Note 16).

(e) *D-Xylose-1-C*^{14}. The crude 1,2-*O*-isopropylidene-D-xylofuranose-1-C^{14} is heated at 100° with 100 ml. of 0.1 *N* sulfuric acid for 1 hour. Acid is removed on an ion exchange column, and evaporation gives a crystalline residue which is triturated with cold 95% alcohol and collected on a filter; yield 2.35 g. (58%), m.p. 144–147° (Note 17).

B. Notes

1. Directions are given by Isbell for preparing the starting material by a modification of the Ruff[1] degradation of calcium D-xylonate dihydrate with peroxide in the presence of ferric acetate catalyst. Neish[2] has reported that the original procedure yields a product containing other reducing substances. Directions are given by Neish for preparing the D-threose by degradation of 1,3-*O*-benzylidene-D-arabitol and hydrolysis of 2,4-*O*-benzylidene-D-threose. Purity of the tetroses was checked by paper chromatography.

2. Neish[2] has reported the yield of ammonia to be 90%.

3. The removal of carbon dioxide is facilitated by agitating the resin (20 ml., Amberlite IR-120H) in a 25-mm. diameter tube.

4. The mother liquors containing lead D-lyxonate-1-C^{14} (b) are saved for the preparation of D-lyxono-γ-lactone-1-C^{14}.

5. A stainless steel 24/40 standard taper stopper supports a stainless steel stirrer, with oilless bearings, which just touches the bottom of a heavy-walled glass tube (200 × 25 mm.) equipped with a side arm (50 × 15 mm.) for addition of amalgam.

6. Trial experiments have shown that the optimum yield of reducing sugar (87%) is obtained by the use of 4.6 g. of the amalgam and 3.2 g. of sodium acid oxalate per mmole of lactone in 20 ml. of water. For the preparation of the amalgam, see D-mannose-1-C^{14}, Note 13.

7. The column consists of Amberlite IR-100H and Duolite A-4, with an additional 5 ml. of Amberlite at the base. Neish[2] has used Amberlite IR-120 and IR-4B in the acid and base forms, respectively, for this purpose.

8. A further small amount of product is recovered from this final mother liquor, following hydrolysis for 3 hours with 10 ml. of 0.05 *N* hydrochloric acid in a boiling water-bath. The acid is removed by passage of the solution through a 10-ml. column of Duolite A-4, and the neutral effluent is evaporated to dryness under reduced pressure. The radiochemical yield is raised to 61% through the use of 0.6 g. of carrier in three portions.

9. The procedure is the same as that described for α-D-xylose-1-C^{14}. Trial experiments have shown that an optimum yield of 76.2% is obtained with the use of 12.8 g. of sodium acid oxalate and 18.4 g. of 5% sodium amalgam per mmole of the lactone in 20 ml. of water. The amount of 4.2% amalgam used is equivalent to this.

10. The synthesis is accomplished without isolating intermediates, as such a procedure raises the over-all yield from 20% to 55–60%.

11. The procedure is adapted from the directions of Mehltretter,[3] which are capable of producing 1,2-*O*-isopropylidene-D-glucose in aqueous solution (93% yield) from D-glucose.

12. 1,2:5,6-Di-*O*-isopropylidene-D-glucose may be isolated in 69.5% yield.[3]

13. Starch-iodide paper indicates the amount of reagent to be about 7.3 g.

14. This hygroscopic compound, prepared[4] in a similar manner with lead tetraacetate as oxidant, boils at 132–136° (0.01–0.02 mm.) The asymmetry of the compound is maintained only by the blocking isopropylidene group, for hydrolysis produces *meso*-xylaric dialdehyde.

15. The hydrogen absorption requires 15–90 hours, depending upon the preparation. Hydrogenation at 75° and 1500 p.s.i. does not alter the yield.

16. The compound has been described by Svanberg and Sjoberg.[5]

17. A systematic degradation of xylose has been described by Brown.[6]

C. Other Preparations

The preparation of D-xylose-1-C^{14} by essentially Method I has been described in detail by Neish;[2] radiochemical yield 22% based on cyanide-C^{14}. The aldonic acids, D-lyxonic-1-C^{14} acid and D-xylonic-1-C^{14} acid,

formed in the ratio of 2.36 to 1, were separated by crystallization of the cadmium xylonate-cadmium bromide double salt (28.4%). The D-xylonic-1-C^{14} acid was lactonized by heating for 24 hours at 100° and 2 mm. in a drying pistol containing phosphorus pentoxide. The reduction of D-xylono-γ-lactone-1-C^{14} (77%) was performed by the use of 9.3 g. of 5% sodium amalgam, 1.4 g. of oxalic acid dihydrate, 1.6 g. of sodium oxalate and 30 ml. of water per mmole of lactone, additional oxalic acid being added to maintain the pH below 4. The formation of some glyoxylic acid by reduction of oxalic acid was noted, but the use of benzoic or acetic acid buffers gave only 35–40% yields of xylose. The yield of D-lyxono-γ-lactone-1-C^{14} was 67%.

The preparation of D-xylose-2-C^{14} by degradation of glucose-2-C^{14} by Method II has been reported by Altermatt;[7] yield 56%. The intermediates were: 1,2:5,6-di-*O*-isopropylidene-D-glucofuranose-2-C^{14}, 1,2-*O*-isopropylidene-D-glucofuranose-2-C^{14} and 5-aldo-1,2-*O*-isopropylidene-D-xylofuranose-2-C^{14}.

[1]O. Ruff and G. Ollendorf, Ber., *33*, 1798 (1900); H. G. Fletcher, Jr., H. W. Diehl and C. S. Hudson, J. Am. Chem. Soc., *72*, 4546 (1950).

[2]A. C. Neish, Can. J. Chem., *32*, 334 (1954).

[3]C. L. Mehltretter, B. H. Alexander, R. L. Mellies and C. E. Rist, J. Am. Chem. Soc. *73*, 2424 (1951)

[4]K. Iwadare, Bull. Chem. Soc. Japan, *16*, 40 (1941).

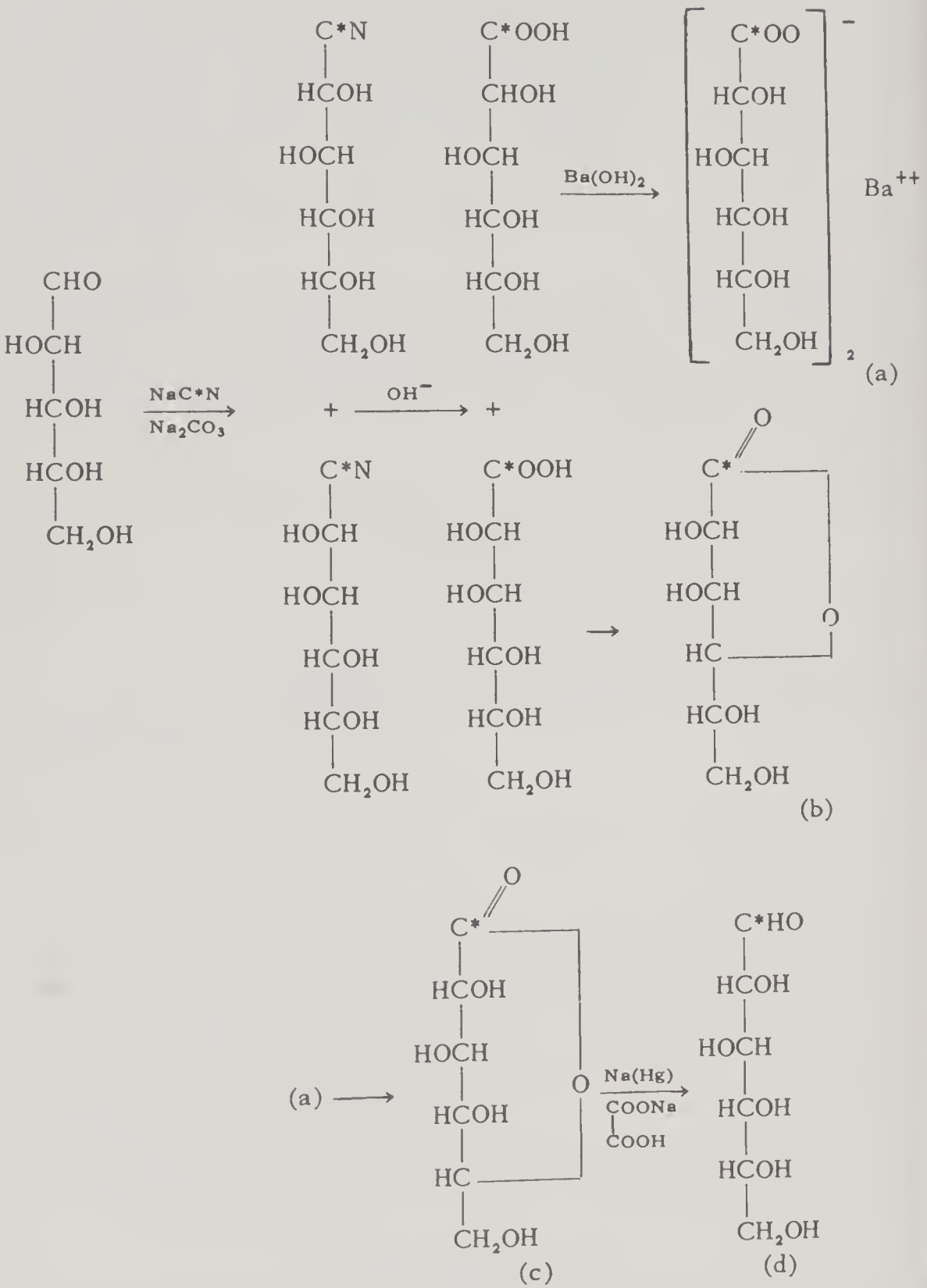
[5]O. Svanberg and K. Sjoberg, Ber., *56*, 863 (1923).

[6]S. A. Brown, Can. J. Biochem. Physiol., *33*, 368 (1955).

[7]H. A. Altermatt, F. J. Simpson and A. C. Neish, Can. J. Biochem. Physiol., *33*, 615 (1955).

D-GLUCOSE-1-C^{14}

METHOD I



H. S. Isbell, J. V. Karabinos, H. L. Frush, N. B. Holt, A. Schwebel and T. T. Galkowski, J. Research Nat. Bur. Standards, *48*, 163 (1952).

A. Procedure (Note 1)

(a) *Barium D-Gluconate-1-C^{14} Trihydrate.* To 10 ml. of a frozen aqueous solution containing 2 mmoles of sodium cyanide-C^{14} and 2 mmoles of sodium hydroxide, in a glass-stoppered tube cooled in a Dry Ice-bath, is added a solution of 2 mmoles of D-arabinose in 10 ml. of 0.2 *M* sodium bicarbonate. The mixture is thawed and stored at room temperature for 48 hours, then heated overnight at 60° in a stream of air to effect hydrolysis. The residue is dissolved in water and passed through a cation exchange column (Note 2). The combined solution and washings are concentrated to about 10 ml. and treated with sufficient barium hydroxide solution to make the solution basic to phenolphthalein. The mixture is digested on a steam-bath for 1 hour, then acidified to phenolphthalein with carbon dioxide and filtered through a bed of diatomaceous earth and decolorizing charcoal. The filtrate and washings are evaporated under reduced pressure to about 1 ml., treated with methanol to the point of incipient turbidity, seeded with barium gluconate trihydrate and stored for several days at room temperature. The crystalline product is collected, washed with cold 25% methanol and dried under vacuum. The first-crop yield of barium gluconate-1-C^{14} trihydrate is 0.268 g., 47.7% on a radiochemical basis. The mother liquor is concentrated in an air stream, treated with 0.5 g. of carrier and diluted with methanol to yield a second crop of 0.549 g., making the total radiochemical yield 64% (Note 3).

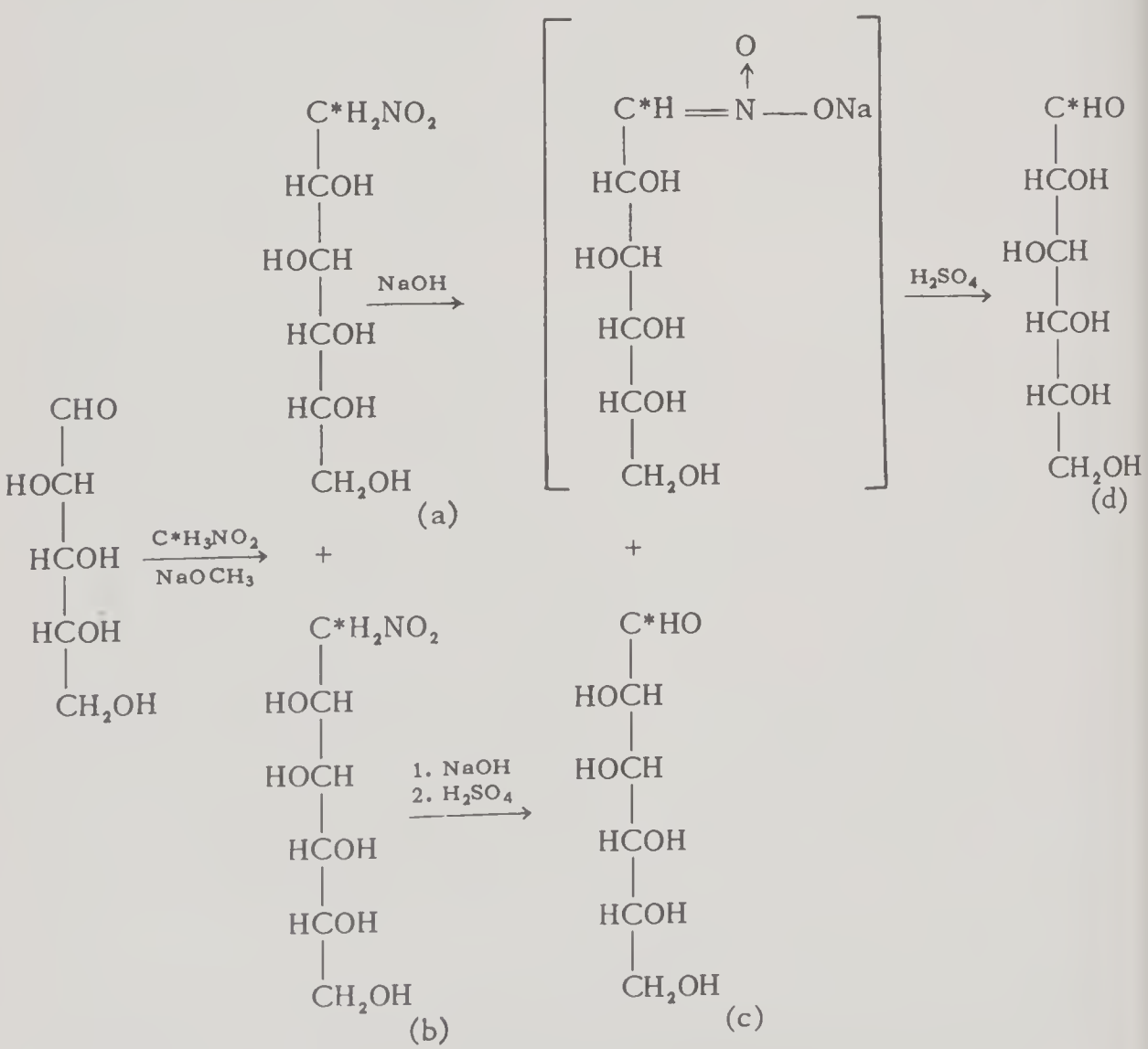
(b) *D-Mannono-γ-lactone-1-C^{14}.* The mother liquor from the synthesis of barium D-gluconate-1-C^{14} is passed through a cation exchange column (Note 4), and the effluent and washings are evaporated under reduced pressure. The residue is diluted with two drops of methanol and 0.5 ml. of isopropyl alcohol, then seeded and stored for 1 week in a stoppered tube. The crystalline product is collected, washed with cold isopropyl alcohol and dried; yield 0.110 g. Treatment of the mother liquor with 0.2 g. of carrier and recrystallization from isopropyl alcohol yield a second crop of 0.18 g., making the total radiochemical yield 30% (Note 5).

(c) *D-Glucono-δ-lactone-1-C^{14}* (Note 6). A solution containing 0.581 g. of barium D-gluconate-1-C^{14} is passed through a cation exchange column (Note 7), followed immediately by water. The combined effluent and washings are lyophilized without delay. The residue is dissolved in methyl cellosolve and seeded, and the solvent is removed in an air stream. The semicrystalline mass is moistened again with solvent, and the evaporation is continued until conversion to the characteristic chunky crystal form is complete (Note 8).

(d) *D-Glucose-1-C^{14}.* To a stirred, ice-cooled mixture of 0.178 g. (1 mmole) of the crude D-glucono-δ-lactone-1-C^{14}, 0.7 g. of oxalic acid, 0.8 g. of sodium oxalate and 20 ml. of water is added 2.3 g. of 5 per cent

sodium amalgam pellets. The mixture is stirred until the amalgam is spent; then the mercury is removed by pipette, three volumes of methanol are added, and the mixture is filtered to remove the precipitated salts, which are washed with methanol and saved for recovery of unreacted gluconic-1-C^{14} acid. The combined solution is neutralized with sodium hydroxide and concentrated to small volume under reduced pressure. The residue is extracted with several portions of methanol (total 10 ml.), and the filtered extract is diluted with water and passed through a deionizing column (see D-mannose-1-C^{14}). The effluent and washings are evaporated under reduced pressure to give 0.184 g. of syrup, which then is dissolved in 4 ml. of methanol. The solution is treated with isopropyl alcohol to the point of incipient turbidity, seeded, and set aside for several days to crystallize; yield 0.144 g. (80%), m.p. 147–149° (Note 9). Degradation studies indicate that there is no isotopic rearrangement (Note 10).

METHOD II



J. C. Sowden, J. Biol. Chem., *180*, 55 (1949); Science, *109*, 229 (1949).

A. Procedure (Note 11)

(a) *1-Nitro-1-deoxy-D-sorbitol-1-C^{14}*, and (b) *1-Nitro-1-deoxy-D-mannitol-1-C^{14}*. To a stirred suspension of 44 g. of D-arabinose in 50 ml. of absolute methanol is added 17.9 g. of nitromethane-C^{14}, followed by a solution of 8.8 g. of sodium in 350 ml. of methanol. The mixture is stirred vigorously at room temperature for 5 hours in a stoppered flask; then the precipitated sodium nitroalcohols are collected on a filter and washed quickly with anhydrous methanol at −20°, ether and petroleum ether. The moist amorphous powder is dissolved in 350 ml. of ice water and passed, at once, through an ion exchange column containing 1 kg. of Amberlite IR-100-AG in the hydrogen form. The effluent (1300 ml.) is evaporated under reduced pressure, and the residual syrup is treated with a few ml. of absolute ethanol. The mixture of crystalline products and unchanged D-arabinose is collected, washed with a small amount of cold ethanol and air-dried; yield, 30 g. (Note 12). The enantiomorphic nitroalcohols are extracted from the mixture with dry acetone in a Soxhlet apparatus for several hours (Note 13). Concentration of the extract and fractional crystallization of the residue from ethanol yield: 1.1 g. of D-arabinose, m.p. 150–160°; 11.0 g. of 1-nitro-1-deoxy-D-mannitol-1-C^{14}, m.p. 130–133°; and 6.1 g. of 1-nitro-1-deoxy-D-sorbitol-1-C^{14}, m.p. 103–105° (Note 14).

(c) *D-Mannose-1-C^{14}* (Note 15). A solution of 5 g. of 1-nitro-1-deoxy-D-mannitol-1-C^{14} in 15 ml. of 2 *N* sodium hydroxide is added dropwise to a stirred solution of 7.5 ml. of sulfuric acid in 9 ml. of water at 10–15°. The solution is diluted to 100 ml. with water, neutralized with sodium carbonate and treated with 3 ml. of phenylhydrazine in 7 ml. of acetic acid. The mixture is stored overnight at 0°, and the precipitated D-mannose-1-C^{14} phenylhydrazone is collected and washed with water, alcohol, and ether; yield 5.0 g. (78%), m.p. 190–191°. A mixture of 4.0 g. of the hydrazone, 50 ml. of water, 10 ml. of alcohol, 5 ml. of benzaldehyde and 0.5 g. of benzoic acid is refluxed for 2.5 hours. The cooled solution is separated from the benzaldehyde phenylhydrazone, then extracted with three portions of chloroform, decolorized with charcoal and evaporated under reduced pressure. The syrup is triturated with absolute alcohol, and the crystalline product is collected by filtration; yield 90%, m.p. 127–131° (Note 16).

(d) *D-Glucose-1-C^{14}* (Note 15). A solution of 3.0 g. of 1-nitro-1-deoxy-D-sorbitol-1-C^{14} in 9 ml. of 2 *N* sodium hydroxide is added dropwise to a stirred solution of 4.5 ml. of sulfuric acid in 5.5 ml. of water at 10–15°. The mixture is diluted with water to 125 ml., neutralized with barium hydroxide to Congo red and filtered. The filtrate is passed successively through two ion exchange columns containing, respectively, 100 g. of Amberlite IR-100-AG and Duolite-A-4 (Note 17). The effluent is evaporated under reduced pressure, and the syrup is crystallized by being

mixed with a few drops of ethanol and warmed at 50°; yield 1.5 g. (58%), m.p. 142–145°. Carrier D-glucose is recrystallized from the mother liquor, yielding 2.0 g. of product, m.p. 145–146°, which contains 14% of the activity of the main crop.

B. Notes

1. See the allied synthesis of D-mannose-1-C^{14}.

2. Amberlite IR-100, 1.2 × 20 cm.

3. Another preparation on the 0.9-mmole scale gave a yield of 71.5%. Barium mannonate does not crystallize.

4. Amberlite IR-100, 1.4 × 30 cm.

5. Another preparation on the 0.9-mmole scale gave a yield of 14.6%. Also see D-mannose-1-C^{14}.

6. Experiment shows that the δ-lactone is reduced more readily than the γ-lactone. The yield of lactone is practically quantitative.

7. A 1 × 30-cm. column of Amberlite IR-100 is conditioned by regeneration, and by washing with water and then (to inhibit the growth of mold) with methanol and water, immediately before use.

8. Purification may be effected by decolorizing with carbon in hot methyl cellosolve-ether mixture. The yield is 90%.

9. Paper chromatography[1] and radioautography of glucose prepared in this manner have disclosed the presence of four radioactive compounds.[2] Purification is accomplished[2] by column chromatography, employing charcoal-Celite[3] with water as eluant. A determination of glucose with anthrone reagent is described by Roe.[4]

10. Degradation by reaction with molecular oxygen in alkaline solution (see potassium D-arabonate-5-C^{14}) forms nonisotopic D-arabonate. Degradations are described by Wood,[5] Topper,[6] Sowden,[7] Abraham,[8] Eisenberg,[9] Bernstein,[10] Landan,[11] Boothroyd,[12] and Perlin.[13] D-glucose-1-C^{14} has been converted[7,8] into formic-C^{14} acid (80–83%) and nonisotopic levulinic acid phenylhydrazone (48%) by treatment with hydrobromic acid, the intermediate possibly being 5-(hydroxymethyl)-2-furaldehyde-C^{14}. Bothner-By[14] has shown that there is no carbon rearrangement during the epimerization of glucose-1-C^{14} in alkaline solution. Sowden[15] has employed the isotope dilution technique in studying the isomerization, fragmentation, and fragment recombination of D-glucose, D-mannose and D-fructose promoted by aqueous alkali.

The interconversion and degradation of D-glucose-2-C^{14} by the action of quaternary ammonium base type anion exchange resins (Amberlite IRA-400) have been described by Buhler.[15a] Among the four radioactive interconversion products were identified: C^{14}-D-glucose, C^{14}-D-fructose and C^{14}-D-mannose; among the six degradation products (three radioactive) were identified: lactic-2-C^{14} acid, glycolic-$C^{14}_{1/2}$ acid and lactyl-

2-C^{14}-lactic-2-C^{14} acid (formed by autoesterification during sample concentration).

11. The procedure is an adaptation of the nitromethane method of Sowden and Fischer[16] for extending the aldose chain. A review of the chemistry of nitroparaffins is presented by Hass.[17]

12. Occasionally subsequent fractionation shows that only 1-nitro-1-deoxy-D-mannitol-1-C^{14} and D-arabinose have crystallized at this stage. In such case, concentration of the mother liquor, followed by the addition of ether, leads to the isolation of relatively pure 1-nitro-1-deoxysorbitol-1-C^{14}.

13. The residue is 10.5 g. of D-arabinose, m.p. 156–159°.

14. Seed crystals of the pure isomers may be used to advantage in the fractional crystallization; the conditions are largely empirical. The bulk of the 1-nitro-1-deoxy-D-mannitol-1-C^{14} and a negligible amount of the starting material crystallize first. Succeeding crops alternate between the more soluble 1-nitro-1-deoxy-D-sorbitol-1-C^{14} and its isomer; the melting points of the pure compounds are 107–108° and 133–134°, respectively.

15. The method is that of Nef.[18] A study of the mechanism of the Nef reaction has been presented by van Tamelen,[19] and the reaction has been studied by Mahler.[20]

For the cyanohydrin method of synthesis see D-mannose-1-C^{14}.

16. D-Mannose-1-C^{14} phenylhydrazone (0.9 g.) is recovered from the mother liquors.

17. The latter column is treated with several volumes of 5% sodium chloride solution and washed chloride-free before use.

C. Other Preparations

By Method I, starting with D-arabinose-1-C^{14} and sodium cyanide, are prepared:[21] barium D-gluconate-2-C^{14} trihydrate (60.3%), D-glucono-δ-lactone-2-C^{14}, α-D-glucose-2-C^{14} (82.8%), and D-mannono-γ-lactone-2-C^{14} (25.4%).

The preparation of D-glucose-1-C^{14} in 10% radiochemical yield has been described by Koshland.[22] The method was a classical Fischer-Kiliani cyanohydrin synthesis, modified by substituting catalytic hydrogenation for the reduction with sodium amalgam, and employing carrier techniques to avoid the brucine salt procedure for the separation of gluconic acid from its epimer, mannonic acid. The same procedure has been employed by Gilvarg;[23] radiochemical yield 7% based on cyanide. By isotope dilution, the product was found to be contaminated with less than 0.5% mannose-1-C^{14}.

The preparation of D-glucose-1-C^{14}, by a modification[24] of Method I, has been reported by Horowitz;[25] radiochemical yield 11%. The prepara-

tion by Method I in excellent yields has been reported by Neish.[26,27] Hers[28] has modified the method by: effecting the cyanohydrin reaction in the presence of calcium chloride; then reducing the mixed epimeric lactones (unreacted hexonic acid was recovered and retreated, contributing 20% of the final sugar), epimerizing the aldose mixture (2.5% hexose w./v.) in 0.2 *M* sodium phosphate (pH 7.5) by heating for 90 minutes on a steam-bath, and finally separating and purifying the products by paper chromatography;[29] over-all yield: D-glucose-1-C^{14} (18.4%), D-mannose-1-C^{14} (2.85%), and D-fructose-1-C^{14} (2.04%). Degradation of the D-glucose-1-C^{14} indicated that there was no isotopic rearrangement.

The preparation of D-glucose-1-C^{14} and D-mannose-1-C^{14}, essentially by Method II, has been reported by Wood[30] (yields 6–7% and 12–15% based on barium carbonate) and Mahler[20] (yields 5% and 7% based on methyl iodide).

The preparation of D-mannose-1-C^{14} by Method II has been reported by Cook.[31] The product was isolated as the phenylhydrazone, under conditions which precluded glucosazone formation,[32] then was regenerated with benzaldehyde. Paper chromatography and radioautography indicated that the product was homogeneous.

Eisenberg[9] has reported the preparation in high yield of potassium D-gluconate-1-C^{14}, -2-C^{14} and -6-C^{14} by oxidation of the corresponding C^{14}-glucose by the potassium hypoiodite-methanol procedure of Moore[33] for oxidizing aldoses to aldonic acids.

The preparation of glucose-2-C^{14} from D-arabinose-1-C^{14} by Method I has been reported by Altermatt,[34] the intermediates being barium D-gluconate-2-C^{14} and D-glucono-δ-lactone-2-C^{14}. The by-product was D-mannono-γ-lactone-2-C^{14}.

In the manner of Method I from L-arabinose have been prepared:[34] L-gluconic-1-C^{14} acid, L-mannonic-1-C^{14} acid, barium L-gluconate-1-C^{14} and L-glucono-δ-lactone-1-C^{14}. The lactone was reduced with sodium borohydride by the method of Wolfrom,[35] forming L-sorbitol-1-C^{14}, (L-glucitol-1-C^{14}), which was converted[34] to D-xylose-5-C^{14} by the method of Hockett.[36] Treatment with furfuraldehyde in the presence of 3 *N* sulfuric acid produced 2,4-*O*-furfurylidene-L-sorbitol-1-C^{14}, which was then oxidized to 2,4-*O*-furfurylidene-D-xylose-5-C^{14} with lead tetraacetate. Hydrolysis yielded D-xylose-5-C^{14}; over-all yield 11% based on potassium cyanide-C^{14}. Frush[37] has described in detail the preparation of calcium L-gluconate-1-C^{14} monohydrate, L-glucose-δ-lactone-1-C^{14} and α-L-glucose-1-C^{14}; yield 52.1% based on sodium cyanide-C^{14}.

The preparation of calcium D-altronate-1-C^{14}, essentially by Method I, has been reported by Neish.[26] D-Ribose was treated with equimolar amounts of potassium cyanide-C^{14} and sodium bicarbonate to effect synthesis of calcium D-altronate-1-C^{14} (44% yield) and D-allono-γ-lac-

tone-1-C^{14} (42% yield). A similar preparation from D-ribose-1-C^{14} and nonisotopic cyanide gave calcium D-altronate-2-C^{14} and crystalline D-allono-γ-lactone-2-C^{14}.

The preparation of crystalline D-allose-1-C^{14} by reduction of D-allono-γ-lactone-1-C^{14}, under the same conditions as were used for D-xylose-1-C^{14} (Method I), has been reported by Neish;[38] yield 75% based on the lactone, 31.5% based on cyanide-C^{14}.

The preparation of potassium D-gluconate-1-C^{14} by oxidation of D-glucose-1-C^{14} with potassium hypoiodite in methanol and its degradation have been reported by Bernstein[10] and Shafizadeh.[39]

The preparation of D-glucose-C^{14}_6-1-phosphate from uniformly labeled starch by the method of Main has been reported.[40]

The preparation of C^{14}-inulin by the cyanohydrin synthesis has been reported.[41]

D-Mannose-1-C^{14} has been converted to D-glucosaccharinic-2,2a-$C^{14}_{1/2}$ acid by the action of lime water at room temperature.[42] Oxidative degradation studies indicate that over 95% of the initial radioactivity is located at C-2a and C-2 in the ratio 2:3. The 1-(2-benzimidazolyl)-1-methyl-C^{14}-1,2,3,4-butanetetrol-1-C^{14} (m.p. 240–241°), formed by condensation with *o*-phenylenediamine (see benzimidazole-2-C^{14}), was oxidized to benzimidazolecarboxylic-C^{14} acid; decarboxylation indicated that nearly 60% of the initial activity was located at C-2 of the saccharinic acid. This result is incompatible with the benzilic acid rearrangement mechanism postulated by Nef[43] and Isbell.[44]

[1]E. F. McFarren, K. Brand and H. R. Rutkowski, Anal. Chem., *23*, 1146 (1951).
[2]F. J. Carleton, S. Misler and H. R. Roberts, J. Biol. Chem., *214*, 427 (1955).
[3]R. L. Whistler and D. F. Durso, J. Am. Chem. Soc., *72*, 677 (1950).
[4]J. H. Roe, J. Biol. Chem., *212*, 335 (1955).
[5]H. G. Wood, N. Lifson and V. Lorber, J. Biol. Chem., *159*, 475 (1945).
[6]Y. J. Topper and A. B. Hastings, J. Biol. Chem., *179*, 1255 (1949).
[7]J. C. Sowden, J. Am. Chem. Soc., *71*, 3568 (1949).
[8]S. Abraham, I. L. Chaikoff and W. Z. Hassid, J. Biol. Chem., *195*, 567 (1952); S. Abraham, E. W. Putnam and W. Z. Hassid, Arch. Biochem. Biophys., *41*, 61 (1952).
[9]F. Eisenberg, Jr., J. Am. Chem. Soc., *76*, 5152 (1954).
[10]I. A. Bernstein, K. Lentz, M. Malm, P. Schambye and H. G. Wood, J. Biol. Chem., *215*, 137 (1955); S. Moore and K. P. Link, J. Biol. Chem., *133*, 293 (1940).
[11]B. R. Landan, A. B. Hastings and F. B. Nesbett, J. Biol. Chem., *214*, 525 (1955).
[12]B. Boothroyd, S. A. Brown, J. A. Thorn and A. C. Neish, Can. J. Biochem. Physiol., *33*, 62 (1955).
[13]A. S. Perlin, J. Am. Chem. Soc., *76*, 2595 (1954).
[14]A. A. Bothner-By and M. Gibbs, J. Am. Chem. Soc., *72*, 4805 (1950).
[15]J. C. Sowden and R. Schaffer, J. Am. Chem. Soc., *74*, 499, 505 (1952).

[15a]D. T. Buhler, R. C. Thomas, B. E. Christensen and C. H. Wang, J. Am. Chem. Soc., 77, 481 (1955).

[16]J. C. Sowden and H. O. L. Fischer, J. Am. Chem. Soc., 69, 1963 (1947); also: 66, 1312 (1944); 67, 1713 (1945); 68, 1511 (1946); 69, 1048 (1947).

[17]H. B. Hass and E. F. Riley, Chem. Revs., 32, 373 (1943).

[18]J. U. Nef, Ann., 280, 263 (1894); J. Johnson and E. F. Degering, J. Org. Chem., 8, 10 (1943).

[19]E. E. van Tamelen and R. J. Thiede, J. Am. Chem. Soc., 74, 2615 (1952).

[20]H. A. Mahler, Atomic Energy Commission Report, AECD-2400; Nuc. Sci. Abstracts, 2, 73 (1949); Nucleonics, 7 (4), 54 (1950).

[21]H. S. Isbell, H. L. Frush and R. Schaffer, Atomic Energy Commission Report, NBS-3004; Nuc. Sci. Abstracts, 8, 2809 (1954).

[22]D. E. Koshland, Jr. and F. H. Westheimer, J. Am. Chem. Soc., 72, 3383 (1950); 71, 1139 (1949).

[23]C. Gilvarg, J. Biol. Chem., 199, 57 (1952).

[24]C. S. Hudson, J. Am. Chem. Soc., 73, 4498 (1951).

[25]H. H. Horowitz, A. P. Doerschuk and C. G. King, J. Biol. Chem., 199, 193 (1952).

[26]A. C. Neish and A. C. Blackwood, Can. J. Biochem. Physiol., 33, 323 (1955); A. C. Neish, Can. J. Chem., 32, 334 (1954); P. A. Levene and W. A. Jacobs, Ber., 43, 3141 (1910).

[27]A. C. Neish and F. J. Simpson, Can. J. Biochem. Physiol., 32, 147 (1954).

[28]H. G. Hers, J. Edelman and V. Ginsburg, J. Am. Chem. Soc., 76, 5160 (1954).

[29]S. M. Partridge, Biochem. J., 42, 238 (1948).

[30]H. G. Wood, Nucleonics, 7 (4), 55 (1950).

[31]M. Cook and V. Lorber, J. Biol. Chem., 199, 1 (1952).

[32]E. C. Sherrard and G. W. Blanco, Ind. Eng. Chem., 15, 611 (1923).

[33]S. Moore and K. P. Link, J. Biol. Chem., 133, 293 (1940).

[34]H. A. Altermatt, F. J. Simpson and A. C. Neish, Can. J. Biochem. Physiol., 33, 615 (1955).

[35]M. L. Wolfrom and H. B. Wood, J. Am. Chem. Soc., 73, 2933 (1951).

[36]R. C. Hockett, U. S. 2,584,129; Chem. Abstracts, 46, 8148 (1953).

[37]H. L. Frush and H. S. Isbell, Atomic Energy Commission Report, NBS-3845; Nuc. Sci. Abstracts, 9, 3117 (1955).

[38]A. C. Neish, Can. J. Biochem. Physiol., 33, 658 (1955).

[39]F. Shafizadeh and M. L. Wolfrom, J. Am. Chem. Soc., 77, 5182 (1955).

[40]P. V. Vittorio, G. Krotkov and G. B. Reed, Can. J. Botany, 32, 369 (1954); A. R. Main, M. A. Thesis, Queen's University, Kingston, Ontario, Canada, 1952.

[41]E. Catlove, M. C. Reder, W. B. Looney, M. Walser and L. W. Mathews, J. Clin. Invest., 33, 924 (1954).

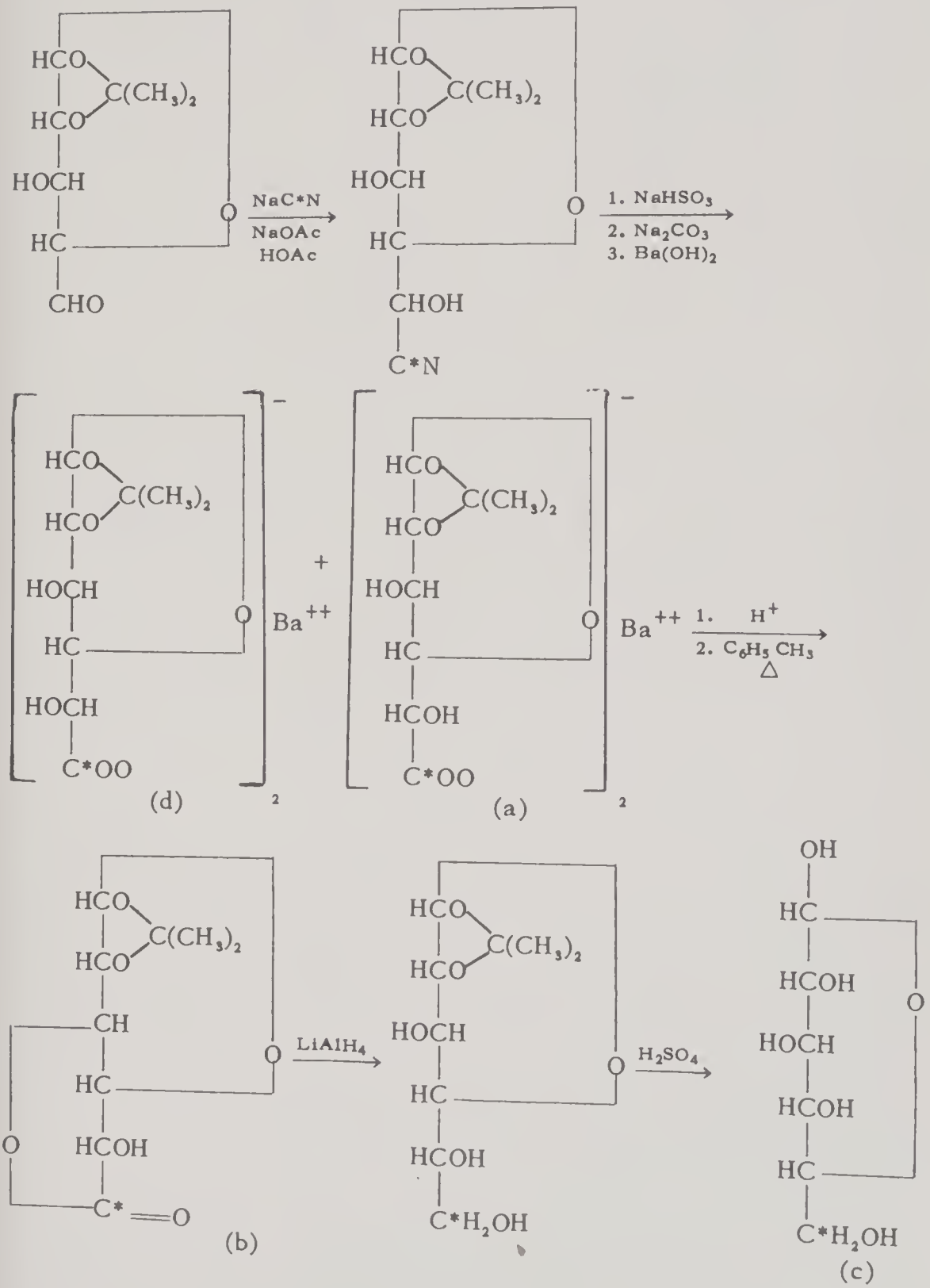
[42]J. C. Sowden and D. J. Kuenne, J. Am. Chem. Soc., 75, 2788 (1953); H. Kiliani, Ber., 15, 701, 2953 (1882); J. Kenner and G. N. Richards, J. Chem. Soc., 1954, 278.

[43]J. U. Nef, Ann., 357, 294 (1907); 376, 1 (1910).

[44]H. S. Isbell, J. Research Natl. Bur. Standards, 32, 45 (1944).

D-GLUCOSE-6-C^{14}

METHOD I



R. Schaffer and H. S. Isbell, Atomic Energy Commission Report, NBS-4084; Nuc. Sci. Abstracts, 9, 7314 (1955); J. Research Nat. Bur. Standards, 56, 191 (1956).

A. Procedure (Note 1)

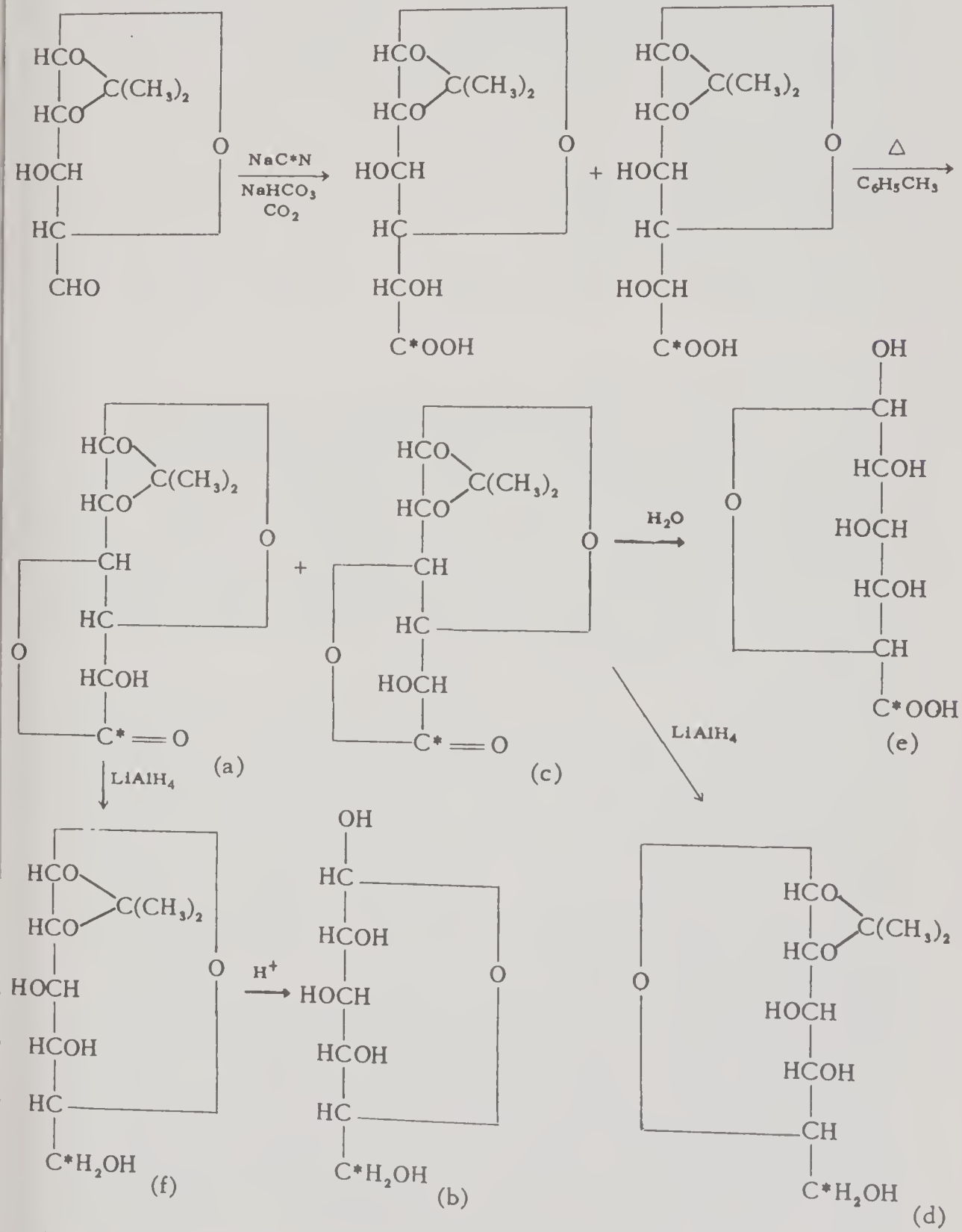
(a) *Barium 1,2-O-Isopropylidene-D-glucuronate-6-C*^{14}. A solution of 1.11 g. (5.9 mmoles) of 1,2-*O*-isopropylidene-D-*xylo*-dialdopentofuranose (Note 2) in 25 ml. of water is frozen on the walls of a 200-ml. flask containing a frozen solution of 5.0 mmoles of sodium cyanide-C^{14} and 5.0 mmoles of sodium hydroxide in 25 ml. of water. Acetic acid (20 ml., 1.0 *N*) is added, and the securely stoppered flask is swirled, until the contents have melted, and then stored at room temperature for 25 days (Note 3). The solution of epimeric nitriles is treated with 0.252 g. of sodium sulfite and heated at 70° for 2 hours (Note 4). The partially hydrolyzed mixture is treated with 1.06 g. of sodium carbonate and refluxed for 2 hours (Note 5). The solution is cooled with an ice-bath and washed through a column of 50 ml. of Amberlite IR-120H (maintained at 0°) with 650 ml. of ice water. The effluent is collected in an ice-cooled flask containing 7.88 g. of crystalline barium hydroxide. The solution is neutralized with gaseous carbon dioxide, filtered, concentrated under reduced pressure, seeded and evaporated under a gentle air stream. The crystalline product (Note 6) is collected on a filter and washed with water and 50% methanol (Note 7). Recrystallization from water gives 0.690 g. of product, 45.6% yield. The radiochemical yield is raised to 54% by the use of three 0.500-g. portions of carrier.

(b) *1,2-O-Isopropylidene-D-glucurono-γ-lactone-6-C*^{14}. A solution of 1.184 g. of the salt in 70 ml. of ice water is passed through an ice-cold column of 9 ml. of Amberlite IR-120H by washing with 130 ml. of ice water. The effluent is frozen and lyophilized. The syrup is refluxed with 100 ml. of toluene for 4 hours, and the filtered solution is evaporated in an air stream; see Other Preparations.

(c) *D-Glucose-6-C*^{14}. To a stirred solution of 13.2 mmoles of lithium aluminum hydride (Note 8) in 30 ml. of absolute ether is added dropwise a solution of the crude lactone in 70 ml. of ether. The mixture is refluxed for 30 minutes and decomposed by the dropwise addition of 25 ml. of absolute ethanol, followed by 130 ml. of water containing 6 ml. of glacial acetic acid. The ether is evaporated in an air stream, and the aqueous solution is washed through a column of 100 ml. of Amberlite IR-120H with ether. The effluent (725 ml.) is concentrated to 200 ml. under reduced pressure, then made 0.1 *N* in sulfuric acid and heated for 2 hours in a boiling water-bath to hydrolyze the 1,2-*O*-isopropylidene-D-glucose-6-C^{14}. The cooled solution is washed through 40 ml. of Duolite A-4 with water, and the effluent is concentrated to 50 ml., filtered through carbon and lyophilized. A filtered solution of the residue in methanol is concentrated to 5 ml., treated with 2-propanol to incipient turbidity, seeded and treated with additional 2-propanol as crystallization proceeds. The product is collected, washed and recrystallized; yield 0.485 g., 69% based on

the barium salt. The radiochemical yield is raised to 84% by the use of 2.0 g. of carrier (Note 9).

METHOD II



F. Shafizadeh and M. L. Wolfrom, J. Am. Chem. Soc., 77, 2568 (1955).

A. Procedure

(a) *1,2-O-Isopropylidene-D-glucurono-γ-lactone-6-C*^{14}. To a solution of 4 g. of 5-aldo-1,2-*O*-isopropylidene-D-xylofuranose (Note 10), 1.2 g. of sodium bicarbonate and several lumps of Dry Ice in 40 ml. of water is added an ice-cooled solution of 0.8 g. of sodium cyanide-C^{14} in 30 ml. of water (Note 11). When the carbon dioxide has evaporated, the mixture is stored at 0° for 2 days and at room temperature for 3 days, then is heated at 60° for 5 hours with aeration and concentration, finally being evaporated under reduced pressure. The residue is boiled briefly with 50 ml. of methanol; then the sodium salts are precipitated by the addition of 100 ml. of ether, collected, washed with ether and dried. A solution of the salts in 20 ml. of water is adjusted to pH 2 with 4 *N* hydrochloric acid and extracted with ten 50-ml. portions of ethyl acetate. The combined extract is dried over sodium sulfate and evaporated (Note 12). The mixture of epimeric acids is refluxed for 3 hours with 40 ml. of toluene, and the clear solution is decanted, cooled and slowly diluted with petroleum ether (b.p. 35–55°) to precipitate the epimeric lactones (1.47 g.). A separation is accomplished by dissolution in 6 ml. of ethyl acetate, dilution with 1.5 ml. of petroleum ether and chromatography on a 2 × 25-cm. column of Florex XXX and Celite (4 : 1). Development is carried out with the same solvent mixture, 5-ml. fractions being collected and evaporated. Fraction 14–20 is recrystallized from ethyl acetate-petroleum ether; further product is obtained from the mother liquor and fraction 13 by rechromatography and recrystallization, making the total yield 0.546 g., m.p. 120° (Note 13).

(b) *D-Glucose-6-C*^{14}. To 18 ml. of clear, stirred ether solution containing 12.8 mmoles of lithium aluminum hydride (Note 9) is added dropwise, under anhydrous conditions, a solution of 1.08 g. of 1,2-*O*-isopropylidene-D-glucurono-γ-lactone-6-C^{14} in 150 ml. of absolute ether during 30 minutes. The addition tube is rinsed with 50 ml. of ether, and the mixture is refluxed for 25 minutes. To the cooled, stirred mixture is added gradually 25 ml. of absolute ethanol (Note 14); then the clear solution is poured into a mixture of 200 ml. of water, 2 ml. of concentrated sulfuric acid and 150 ml. of petroleum ether. The ether phase is separated and washed with 50 ml. of 0.2 *N* sulfuric acid. The combined aqueous solution is heated on a steam-bath for 1.5 hours, cooled and treated with an excess of barium carbonate. The precipitate is separated by centrifuging and washed with three portions of water and finally with boiling water. The solution is passed through layers of ion exchange resin: IR-120H (20 ml.), IR-4B (20 ml.) and IR-120H (10 ml.). The combined effluent and washings (Note 15) are concentrated, and the colorless syrup is diluted with 4 ml. of 95% alcohol, seeded and stored for 1 week; yield 0.72 g., m.p. 146°, $[\alpha]_D^{24}$ 52.3°, equilibrium in water.

(c) *1,2-O-Isopropylidene-L-idurono-γ-lactone-6-C*^{14}. The combined residue from fractions 10–12 (see a, above) is recrystallized from acetone-petroleum ether; yield 0.568 g. (Note 16), m.p. 137–138°, $[\alpha]_D^{26}$ + 91° (*c* 1.82, acetone).

(d) *1,2-O-Isopropylidene-L-idose-6-C*^{14}. 1,2-*O*-Isopropylidene-L-idurono-γ-lactone-6-C^{14} (0.230 g.) is reduced with lithium aluminum hydride, and the product is recrystallized from ethyl acetate; yield 0.178 g., m.p. 113–114°, $[\alpha]_D^{26}$ −20° (*c* 2.7, methanol).

(e) *L-Iduronic-6-C*^{14} *Acid*. A mixture of 1.0 g. of 1,2-*O*-isopropylidene-L-idurono-γ-lactone-6-C^{14}, 25 ml. of water and 5 ml. of Amberlite IR-120H is heated for 3 hours on a steam-bath. The filtered solution is evaporated under reduced pressure, and the residue is crystallized from methanol-ethyl acetate. The yield of recrystallized product is 0.3 g., m.p. 131–132°, $[\alpha]_D^{22}$ +37° (3.5 min.) → +33° (28 min., 4 hr.) (*c* 3, water) (Note 17).

B. Notes

1. The procedure is an improvement upon the isotopic methods of Sowden,[1] Roseman[2] and Shafizadeh (see Method II).

2. Directions are given by Schaffer and Isbell for preparing the starting material in crystalline form, thereby eliminating the drawbacks inherent in the syrupy preparations. C-6 of D-glucose is removed as formaldehyde by the cleavage of 1,2-*O*-isopropylidene-D-glucofuranose with sodium metaperiodate[3] (see D-xylose-1-C^{14}). The excess of this syrupy starting material that can be used in the condensation is limited by the fact that the syrup tenaciously retains part of the formaldehyde, which leads to the formation of glycolic-1-C^{14} acid. Appreciable amounts of unreacted starting material also give rise to troublesome sugar destruction products during the subsequent hydrolysis. The lyophilized concentrate is extracted with chloroform, and the extract is clarified with charcoal and evaporated. The residue is dissolved in water, and the filtered solution is concentrated and stored in a refrigerator for several weeks. The dimer hemihydrate, m.p. 180–184°, is converted to the anhydrous compound, m.p. 182–184°, by recrystallization from boiling benzene.

3. Under highly alkaline conditions more than one mole of cyanide reacts with each mole of the dialdehyde, and the mixture contains dark-colored products. The optimum conditions were arrived at through tracer studies and analysis of the products by isotope dilution technique. In acid solution the reaction is slow, but yield is high.

4. There is considerable decomposition under the conditions usually used for hydrolyzing nitriles in the cyanohydrin synthesis. The sulfite gives a lighter colored solution.

5. The course of hydrolysis may be followed by titration of the ammonia evolved.

6. Directions are given by Schaffer and Isbell for converting the barium salt to sodium D-glucuronate-6-C^{14} monohydrate[4] (92% radiochemical yield) and D-glucurone-6-C^{14}, (D-glucuronolactone-6-C^{14}) (93% radiochemical yield). The free acid is obtained by heating with Amberlite IR-120H, which also hydrolyzes the isopropylidene group. The lactone is obtained by lyophilization of the aqueous acid and crystallization of the residue in methyl cellosolve. A degradation of glucuronic acid is described by Eisenberg.[5]

7. The mother liquor contains the noncrystalline epimer, barium 1,2-*O*-isopropylidene-L-iduronate-6-C^{14}, (d). Directions are given by Shafizadeh for isolating the epimeric by-products; see Method II.

8. Directions are given by Brown[6] for preparing and assaying solutions of lithium aluminum hydride.

9. The over-all radiochemical yield is 48% based on sodium cyanide-C^{14}.

10. The starting material was the syrup rather than the crystalline form (Note 2).

11. In the presence of sodium bicarbonate-carbon dioxide a higher proportion of L-idurono derivative is formed, whereas in the presence of sodium carbonate the proportion of D-glucurono derivative is increased. In the latter case, however, the over-all yield of epimeric products is lower. See D-arabinose-1-C^{14}, Note 1.

12. 1,2-*O*-Isopropylidene-D-glucuronic acid crystallizes readily from the mixture, but purification by recrystallization is a wasteful process, the recovery of remaining material requiring the use of isotopic dilution at the lactone stage.

13. The yield is only 0.204 g. when the sodium bicarbonate is replaced by 1.5 g. of sodium carbonate.

14. Directions are given by Roseman[2] for isolating the intermediate (f), 1,2-*O*-isopropylidene-D-glucose-6-C^{14}, by deionizing the acidified aqueous solution with IR-120H and IR-4B and concentrating the neutral solution; yield of crude product 1.05 g., m.p. 153–157°. Recrystallization from ethyl acetate gives 0.70 g. of colorless product, m.p. 161–162°, $[\alpha]_D^{24} - 12.0°$ (*c* 5.4, water).

15. The solution is negative toward the naphthoresorcinol-uronic acid test and contains 0.85 g. of glucose, according to quantitative anthrone and reducing sugar analyses.

16. The yield is only 0.121 g. under the conditions of Note 2.

17. The product is acid to litmus and gives a strong naphthoresorcinol test for uronic acids.

C. Other Preparations

The preparation of D-glucose-6-C^{14} (10% over-all radiochemical yield) by a modification of Method I has been described by Sowden.[1] 1,2-*O*-Iso-

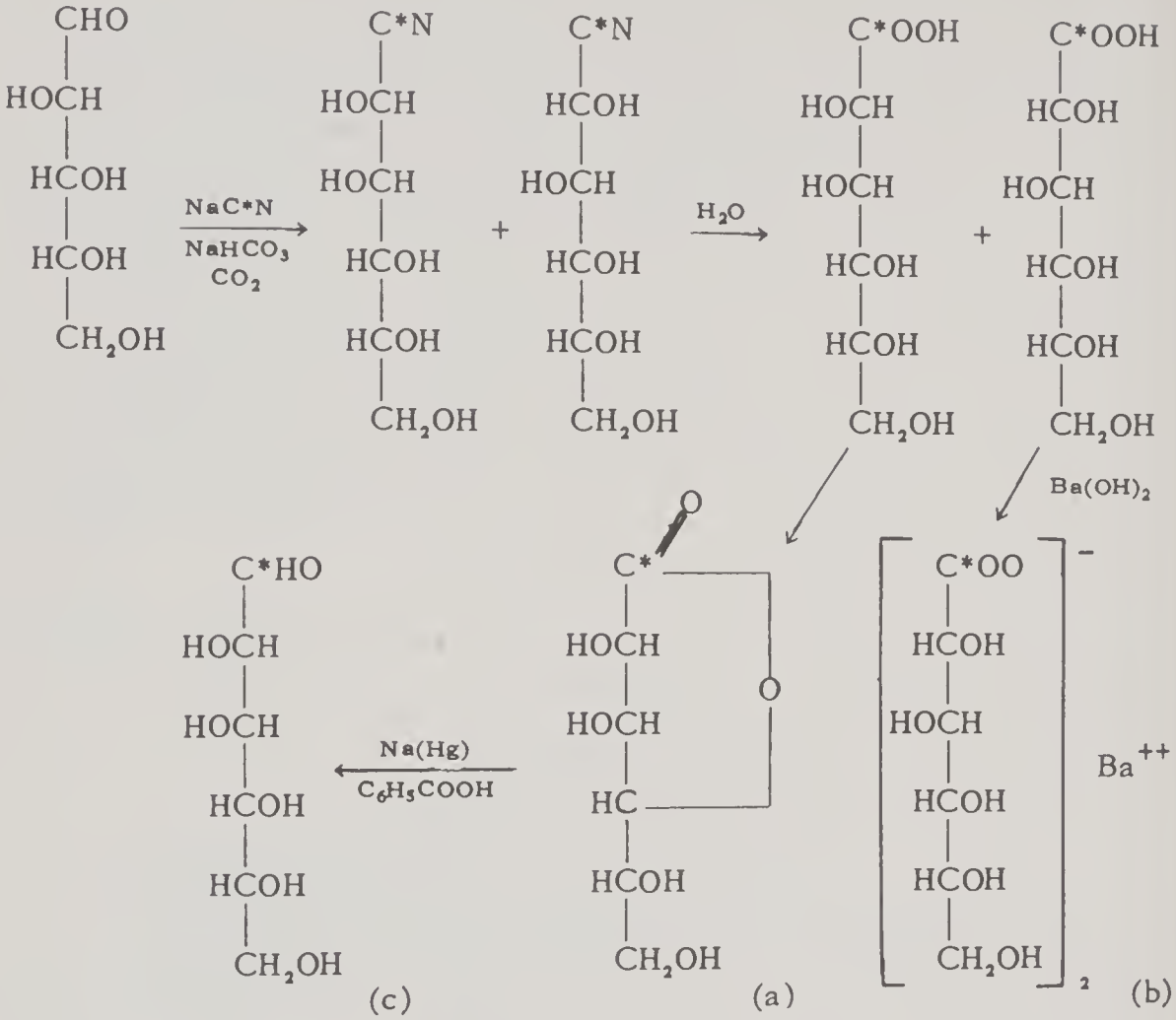
propylidene-D-glucuronic-6-C^{14} acid, prepared by condensation of sodium cyanide-C^{14} with 5-aldo-1,2-*O*-isopropylidene-D-xylofuranose in aqueous solution for 70 hours at 0° (radiochemical yield 12.5%, m.p. 143–144° from acetone-petroleum ether), was lactonized as described (yield 98%, m.p. 119–120° from toluene-petroleum ether). The lactone was reduced in aqueous solution with sodium borohydride[7] and hydrolyzed with sulfuric acid. The solution was deionized with Duolite A-4 and Amberlite IR-100H, and the D-glucose-6-C^{14} was crystallized from 95% alcohol; radiochemical yield 60%, m.p. 145–146°, $[\alpha]_D$ 52.5° equil. in water.

Roseman[2] has reported difficulty in completely removing borate ion in this manner. D-Glucuronolactone-6-C^{14} is prepared[1,8] by heating a solution of 0.100 g. of 1,2-*O*-isopropylidene-D-glucuronic-6-C^{14} acid in a few ml. of water for 2 hours on a steam-bath. The mixture is evaporated to dryness, and the residue is triturated with a few drops of alcohol and seeded; yield 0.075 g. (100%), m.p. 168–172°.

The preparation of D-glucose-6-C^{14} by a combination of the methods of Sowden[1] amd Roseman[2] has been reported by Katz[9] and Abraham.[10] Purity was checked by paper chromatography.[11]

[1]J. C. Sowden, J. Am. Chem. Soc., *74*, 4377 (1952).
[2]S. Roseman, J. Am. Chem. Soc., *74*, 4467 (1952).
[3]J. C. Sowden, J. Am. Chem. Soc., *73*, 5496 (1951).
[4]W. Hach and D. G. Benjamin, J. Am. Chem. Soc., *76*, 917 (1954).
[5]F. Eisenberg, Jr. and S. Gurin, J. Am. Chem. Soc., *73*, 4440 (1951).
[6]*Organic Reactions*, Vol. VI, Wiley, New York, 1951, p. 484.
[7]M. Abdel-Akher, J. K. Hamilton and F. Smith, J. Am. Chem. Soc., *73*, 4691 (1951); M. L. Wolfrom and K. Anno, J. Am. Chem. Soc., *74*, 5583 (1952).
[8]L. Zervas and P. Sessler, Ber., *66*, 1326 (1933).
[9]J. Katz, S. Abraham, R. Hill and I. L. Chaikoff, J. Biol. Chem., *214*, 853 (1955).
[10]S. Abraham, R. Hill and I. L. Chaikoff, Cancer Research, *15*, 177 (1955).
[11]J. Katz and I. L. Chaikoff, J. Biol. Chem., *206*, 887 (1954).

D-MANNOSE-1-C^{14}



H. S. Isbell, J. V. Karabinos, H. L. Frush, N. B. Holt, A. Schwebel and T. T. Galkowski, J. Research Nat. Bur. Standards, *48*, 163 (1952).

A. Procedure (Note 1)

(a) *D-Mannono-γ-lactone-1-*C^{14} (Note 2). To a mixture of 2 mmoles of sodium cyanide-C^{14} and 2.24 mmoles of sodium hydroxide in 5 ml. of solution, frozen in a small flask at 78°, is added 1 g. of solid carbon dioxide and 20 ml. of a solution containing 2 mmoles of D-arabinose. The mixture is thawed, and the flask is stoppered and stored overnight in a refrigerator, then at room temperature for 2 days (Note 3). The nitriles are hydrolyzed by heating the solution at 50° for 4 hours under reduced pressure, then for 1 hour on a steam-bath (Note 4). The aqueous solution is passed through a cation exchange column (Note 5), and the effluent and washings are lyophilized. The residual syrup is moistened with methanol, seeded with D-mannono-γ-lactone and stored for 3 days (Note 6). The semicrystalline mass is dissolved in 0.5 ml. of methyl cellosolve, treated with ether to incipient turbidity, seeded and set aside for

several days. The crystals are collected, washed with cold ethanol and with ether, and dried at 60° in a vacuum oven; yield 0.192 g., m.p. 151–152°. The radiochemical yield is 54%. The residual solutions are combined, evaporated and diluted with 0.180 g. of carrier. The residue is dissolved in 1 ml. of methyl cellosolve, treated with ether to incipient turbidity and set aside to crystallize; total radiochemical yield 67% (Note 7).

(b) *Barium D-Gluconate-1-C^{14} Trihydrate* (Note 8). To the combined and evaporated mother liquor and washings from the crystallization of D-mannono-γ-lactone-1-C^{14} are added 0.315 g. (1 mmole) of barium hydroxide octahydrate and 3 ml. of water. The mixture is heated for 1 hour on a steam-bath, acidified to phenolphthalein with carbon dioxide and filtered through a bed of diatomaceous earth and decolorizing charcoal; then the combined filtrate and washings are lyophilized. The residue is dissolved in 0.5 ml. of warm water, and the solution is treated with methanol to the point of turbidity and seeded with barium gluconate trihydrate (Note 9). After a few days the product is separated, by removing the mother liquor with a capillary pipette, washed with cold 25% methanol and dried under vacuum; yield 0.042 g. (7.2% on a radiochemical basis). An additional 0.149 g. of product is obtained by working up the residues with 0.100 g. of carrier, making the total radiochemical yield 19.4% (Note 10).

(c) *D-Mannose-1-C^{14}*. The reduction is performed in a heavy-walled glass tube fitted with a bearing and a stirrer made by flattening one end of a stainless steel rod (Note 11). To a suspension of 1.5 g. of benzoic acid (Note 12) in a solution of 0.178 g. (1 mmole) of D-mannono-γ-lactone-1-C^{14} in 20 ml. of water is added 4.6 g. of 5% sodium amalgam pellets (Note 13), and the mixture is stirred for 1.5 hours at 0°. The mercury is removed by pipette, 1.2 g. of benzoic acid and 4.6 g. of sodium amalgam are added, and stirring is continued for 2 hours. The mercury is removed, 1.26 g. of oxalic acid dihydrate is added, and the liberated benzoic acid is removed by chloroform extraction. The aqueous solution is diluted with 2 volumes of methanol and 2 volumes of ethanol, and the precipitated sodium salts are collected, washed with methanol and saved for recovery of unreacted mannonic-1-C^{14} acid. The combined solution is concentrated to 10 ml. under reduced pressure, and the mixed salts are precipitated further by dilution with the alcohols. The filtered solutions are concentrated to 5 ml. and passed through a deionizing column (Note 14). The aqueous solution and washings are combined and lyophilized. The residue is dissolved in 4 ml. of methanol, and the solution is diluted with isopropyl alcohol to the point of incipient turbidity, seeded and stored for several days to crystallize; yield 0.099 g. (55%), m.p. 130–132°. The crystallization of 0.200 g. of carrier from the mother

liquor gives a recovery of 0.180 g. of a more dilute product, raising the radiochemical yield to 74.6%.

B. Notes

1. The procedure is a modification of the Fischer-Kiliani cyanohydrin synthesis[1] (also see Koshland and Westheimer[2]), based upon a thorough study of the reaction conditions. D-Mannose-1-C^{14} is obtained as a by-product in the preparation of D-glucose-1-C^{14}; see Method II.

The relatively small number of carbonyl groups in large polysaccharide molecules may be determined[3] by utilizing this reaction with isotopic cyanide. Hydrolysis results in formation of a molecule containing a carboxyl group, permitting ready separation and purification by means of ion exchange resins.

2. The proportion of the epimers formed may be controlled by varying the conditions of the condensation of D-arabinose with cyanide. The ratio of gluconate to mannonate, as determined by measurement of optical rotation, is altered from 72:28 to 30:70, by changing the added salt from calcium chloride to sodium bicarbonate-carbon dioxide.

3. The condensation is practically quanitative, as determined by titration of residual cyanide in trial runs.

4. Hydrolysis may also be effected by heating at 60–70° under the same conditions, or overnight at 60° in a stream of air. The evolution of ammonia is nearly quantitative under these mild conditions.

5. Amberlite IR-100, 1.4 × 24 cm.

6. The reduction of aldonic acids takes place only through the lactones. Experiment shows that the best results are obtained with mannono-γ-lactone and glucono-δ-lactone. The former compound may be crystallized satisfactorily by concentrating a solution of the epimeric acids in methanol, methyl cellosolve or acetic acid at room temperature in the presence of seed crystals. Recrystallization from hot isopropanol gives a pure product.

7. A preparation at the 3.5-mmole scale gave a yield of 62.5%.

8. The epimeric acids are separated most satisfactorily by the more facile crystallization of the mannono-γ-lactone from concentrated solution and precipitation of barium (or lead) gluconate trihydrate from the mother liquors. Mannonic acid does not form such a salt.

9. Several months time is required sometimes for crystallization in the absence of seed crystals.

10. A preparation at the 3.5-mmole scale gave a yield of 22.3%.

11. A diagram of the apparatus is presented by Isbell. A side arm provides a convenient means of introducing the amalgam.

Two macro methods for the efficient reduction of 5–10 g. of lactone are described by Frush.[4] One employs a stainless-steel flask equipped

with a stirrer that sweeps the bottom, the other a high-speed blender. The former method requires two or three times as much amalgam as does that with the blender.

12. As the reduction of aldonic acid lactones with sodium amalgam takes place only in mildly acid solution, the required pH of the medium at the millimole scale is maintained by use of an excess of a difficultly soluble organic acid or acid salt. Experiment has shown that the conditions required for maximum yield vary with the lactone. The newer procedure[5] employs sodium acid oxalate buffer, eliminating the troublesome removal of benzoic acid; see D-arabinose-1-C^{14} or α-D-xylose-1-C^{14} (Method I). Trial experiment has shown that the optimum yield of reducing sugar (91.6%) is obtained, in this case, by the use of 12.0 g. of sodium acid oxalate and 27.6 g. of 5% sodium amalgam pellets per mmole of lactone in 20 ml. of water at 0°. The amalgam is added in one-third portions at hourly intervals.

13. The efficiency of the reaction is due partly to the uniformity and form of the amalgam, which is prepared by pouring the melt through a heated alundum thimble, with a small hole in the bottom, into a 70-cm. column of mineral oil. The pellets are blotted dry, weighed and rinsed with benzene prior to use.

14. The 1.4 × 10-cm. column contains equal parts of Amberlite IR-100 and Duolite A-4.

C. Other Preparations

See D-glucose-1-C^{14}, Method II (c).

α-D-Mannose-2-C^{14} is prepared[1] by the newer procedure (Note 12) from D-mannono-γ-lactone-2-C^{14} in 69.7% radiochemical yield.

[1]H. Kiliani, Ber., *19*, 3029 (1886); *20*, 282, 339 (1887); E. Fischer, *ibid.*, *22*, 2204 (1889).

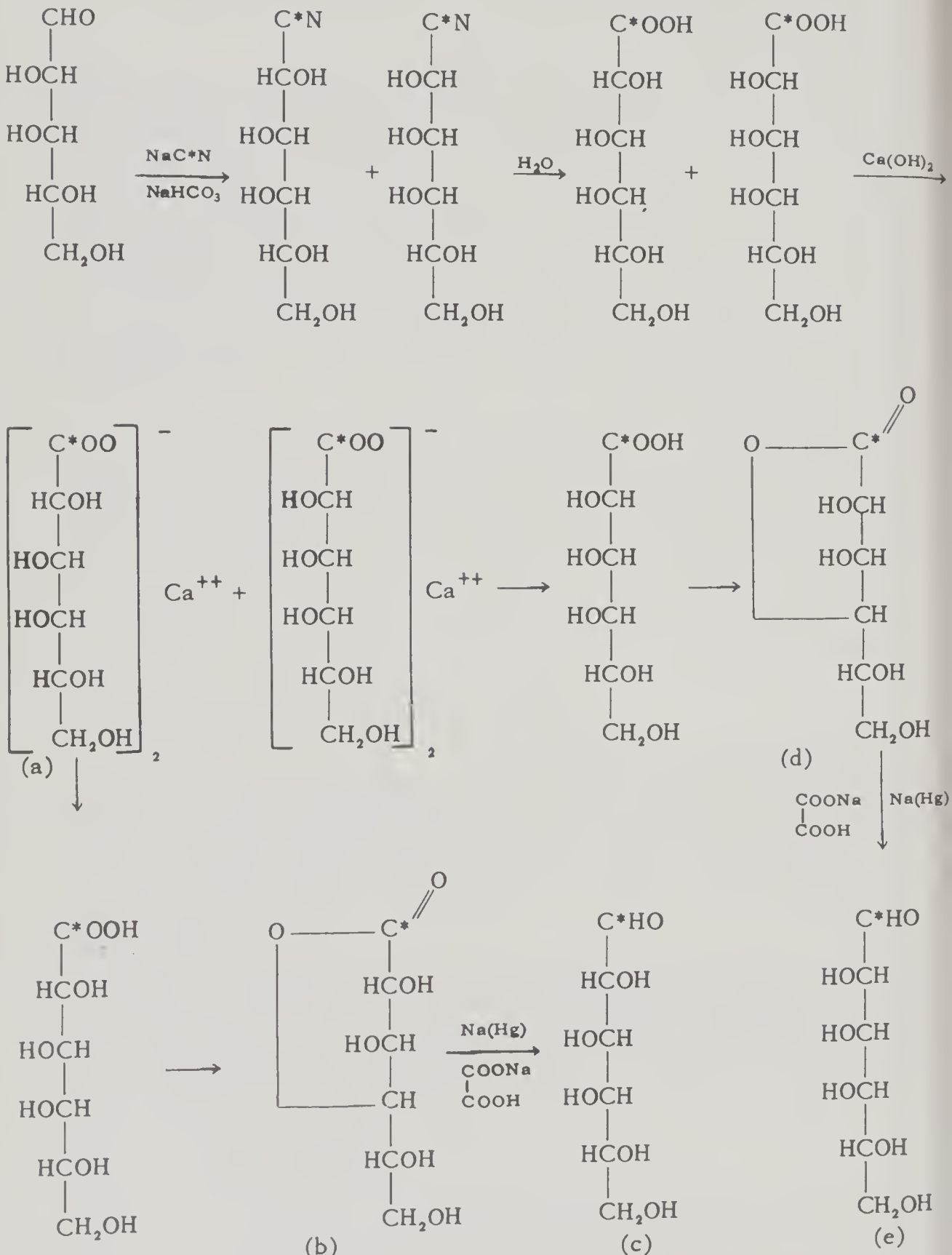
[2]D. E. Koshland, Jr., and F. H. Westheimer, J. Am. Chem. Soc., *72*, 3383 (1950).

[3]H. S. Isbell, Science, *113*, 532 (1951).

[4]H. L. Frush and H. S. Isbell, Atomic Energy Commission Report, NBS-3845; Nuc. Sci. Abstracts, *9*, 3117 (1955).

[5]H. S. Isbell, H. L. Frush and R. Schaffer, Atomic Energy Commission Report, NBS-3004; Nuc. Sci. Abstracts, *8*, 2809 (1954).

α-D-GALACTOSE-1-C^{14}



H. S. Isbell, H. L. Frush and N. B. Holt, J. Research Nat. Bur. Standards, *53*, 217 (1954).

A. Procedure

(a) *Calcium D-Galactonate-1-C^{14} Pentahydrate.* To a 100-ml. round-bottomed flask containing a frozen mixture of 20 ml. of water, 10 mmoles of sodium hydroxide and 12 mmoles of sodium cyanide-C^{14} is added a carbon dioxide-saturated solution containing 10 mmoles of sodium bicarbonate, 13 mmoles of D-lyxose (Note 1) and 10 ml. of water. The flask is stoppered and shaken gently until the ice is melted, then is stored for 5 days at 0°. The mixture of epimeric nitriles is hydrolyzed by heating at 80° under an air-stream, with occasional addition of water until ammonia is no longer evolved (12 hours). The solution is diluted with 25 ml. of water and passed through a cation exchange resin (Note 2). The combined effluent and washings are concentrated to 100 ml. under reduced pressure and then made just alkaline to phenolphthalein with a suspension of calcium hydroxide. The filtered solution is concentrated to a syrup, treated with methanol, seeded and stored for 3 days. The product is collected and recrystallized from a minimal volume of hot water by dilution with methanol; yield 1.838 g. (7.07 mmoles). The radiochemical yield is raised from 59.2% to 66.9% by the use of four 0.7-g. portions of carrier (Note 3).

(b) *D-Galactono-γ-lactone-1-C^{14}.* A solution of 1.42 g. of calcium D-galactonate-1-C^{14} pentahydrate in 100 ml. of water is passed through a column of cation exchange resin (Note 2). The combined effluent and washings are concentrated under reduced pressure to 25 ml., then distributed equally to 5 reduction tubes (Note 4). Lactonization of the D-galactonic-1-C^{14} acid is effected by evaporating the solutions under an air-stream at a bath temperature of 80°, then dissolving the residue from time to time in a little methyl cellosolve and continuing the evaporation for 3 days (Note 5).

(c) *α-D-Galactose-1-C^{14}.* To each reduction tube, containing a vigorously stirred, ice-cooled mixture of 1.1 mmoles of the lactone, 2.3 g. of sodium acid oxalate and 20 ml. of ice water, is added 5.0 g. of 5% sodium amalgam pellets in one portion (Notes 4,6). Stirring is continued until the amalgam is completely decomposed (2 hours); then the mercury is separated, and the combined solution is diluted with 5 volumes of methanol and filtered. The filtrate is concentrated under reduced pressure to 20 ml., then cooled to 0° and made just basic to phenolphthalein with sodium hydroxide. The mixture is diluted with an equal volume of methanol and filtered, and the filtrate is concentrated to 15 ml. and diluted with 2 parts of methanol. The third crop of sodium salts is separated, and the filtrate is evaporated to remove methanol. The residue is dissolved in water and passed through a deionizing column (Note 7). The combined effluent and washings are concentrated under reduced pressure to 20 ml.; then the solution is passed through a slender column of

decolorizing carbon (0.1 g.), and the filtrate is lyophilized. Crystallization of the product is effected by dissolution of the residue in 3 ml. of methanol, addition of 2-propanol to the point of incipient turbidity, and storage for 3 days. The mother liquor is pipetted off, and the crystals are washed with ethanol. The product is recrystallized from 1 ml. of warm water by dilution with 5 ml. of methanol and saturation with 2-propanol; yield 0.760 g. The radiochemical yield is raised, from 77.2% to 85.6% based on the calcium salt, by recrystallizing three 0.5-g. portions of carrier from the combined mother liquors. The radiochemical yield is 98.3% based on unrecovered calcium salt (Note 8).

(d) *D-Talono-γ-lactone-1-C*^{14}. A solution of the combined residual liquors (25 ml. water), containing calcium D-talonate-1-C^{14} from the preparation of calcium D-galactonate-1-C^{14} pentahydrate, is passed through a column containing 25 ml. of Amberlite IR-120H. The combined effluent and washings are concentrated under reduced pressure, and the D-talonic-1-C^{14} acid is transferred to a test tube with methyl cellosolve. The acid is heated at 60° in an air stream for 8 hours, a few drops of the solvent being added at 2-hour intervals. A solution of the residue and 0.2 g. of carrier in 5 ml. of 2-propanol is seeded and stored for 24 hours. The yield of once-recrystallized product is 0.606 g.; radiochemical yield 21.7%. The total radiochemical yield is raised to 28% by treating the combined mother liquors with two 0.2-g. portions of carrier.

(e) *D-Talose-1-C*^{14}. D-Talono-γ-lactone-1-C^{14} (0.430 g.) is reduced in two portions by use of 7.5 g. of sodium acid oxalate and 13 g. of 4.2% sodium amalgam per portion (Note 9) in the manner described above (c). A solution of the lyophilized residue in 0.5 ml. of ethanol is treated with 1-butanol to the point of incipient turbidity, seeded (Note 10) and stored for several days. The yield of product, once recrystallized from the minimum amount of water by dilution with 2-propanol, is 0.170 g. The radiochemical yield is raised from 37.5% to 56% by the use of 0.6 g. of carrier in two portions.

B. Notes

1. The preparation of the starting material is described by Bates.[1] General acid catalysts favor formation of the galactonic epimer in the cyanohydrin synthesis with D-lyxose; see D-mannose-1-C^{14}.

2. Amberlite IR-120H, analytical grade, 70 ml.

3. The combined mother liquors containing calcium D-talonate-1-C^{14} are saved for the preparation of D-talono-γ-lactone-1-C^{14}.

4. See D-mannose-1-C^{14} and α-D-xylose-1-C^{14} (Method I) for the preparation of the amalgam and description of the reduction tubes.

5. The needle-like crystals of the acid are slowly converted to the wedge-shaped crystals of the product. When the reaction appears to be complete, the tubes are stored in a desiccator until wanted.

6. Trial experiments have shown that the optimum yield (95.3%) is obtained with the use of 2.0 g. of sodium acid oxalate and 4.6 g. of 5% sodium amalgam per mmole of the lactone in 20 ml. of water.

7. The column contains 50 ml. of a mixture of equal parts of Amberlite IR-100H and Duolite A-4, being bounded at the top and bottom by 5 ml. of the Amberlite cation exchange resin.

8. Paper chromatography and radioautography have indicated that this product is radioactively homogeneous.[2]

Extraction of the mixed resin column with 250 ml. of 10% acetic acid gives a recovery of 12% of the activity of the calcium D-galactonate-1-C^{14} pentahydrate. Evaporation of the extract to dryness, treatment with carrier, and working up as in procedure (a) give a recovery of 10% unreacted starting material.

9. Trial experiments have shown that the optimum yield of reducing sugar (75–76%) is obtained by the use of 4.6–9.2 g. of 5% sodium amalgam and 3.2–6.4 g. of sodium acid oxalate per 0.5 mmole of this lactone in 20 ml. of water.

10. The preparation of α-D-talose has been described by Pigman.[3]

C. Other Preparations

In the same manner, starting with crude β-D-lyxose-1-C^{14} and sodium cyanide are prepared:[4] calcium D-galactonate-2-C^{14} pentahydrate (46.5% radiochemical yield based on 3 mmoles of D-lyxono-γ-lactone-1-C^{14}), D-galactonic-2-C^{14} acid, D-galactono-γ-lactone-2-C^{14} and α-D-galactose-2-C^{14} (77.3% radiochemical yield based on the calcium salt taken).

The preparation of D-galactose-1-C^{14} in 21.3% radiochemical yield from sodium cyanide-C^{14} has been reported by Topper.[5] D-Lyxose was condensed with 8.2 mmoles of hydrogen cyanide-C^{14} according to the method of Fischer,[6] and D-galactonic-1-C^{14} acid was isolated in 32% yield as the cadmium salt.[6] The D-galactono-γ-lactone-1-C^{14} (89% yield, m.p. 134–136° from absolute alcohol) was reduced to the sugar with sodium amalgam by the method of Sperber;[7] yield 76%, m.p. 168–170° from aqueous methanol. The product was shown by paper chromatography to be free from appreciable isotopic contamination.

D-Galacto-α-metasaccharinic-1-C^{14} acid has been prepared[8] from D-galactose-1-C^{14} by the action of saturated lime-water at room temperature. The 1-(2-benzimidazolyl-2-C^{14})-1,3,4,5-pentanetetrol (m.p. 186–187°), prepared by condensation with *o*-phenylenediamine, was oxidized to benzimidazole-2-C^{14} containing about 95% of the initial radioactivity. This result is compatible with the benzilic acid rearrangement mechanism of Nef and Isbell (see D-glucosaccharinic-2,2a-$C^{14}_{1/2}$ acid).

[1] F. J. Bates, NBS Circular 440, 469 (1942).

[2] F. J. Carleton, S. Misler and H. R. Roberts, J. Biol. Chem., *214*, 427 (1955); E. F. McFarren, K. Brand and H. R. Rutkowski, Anal. Chem., *23*, 1146 (1951).

[3]W. W. Pigman and H. S. Isbell, J. Research Nat. Bur. Standards, *19*, 189 (1937).

[4]H. S. Isbell, H. L. Frush and R. Schaffer, Atomic Energy Commission Report, NBS-3004; Nuc. Sci. Abstracts, *8*, 2809 (1954).

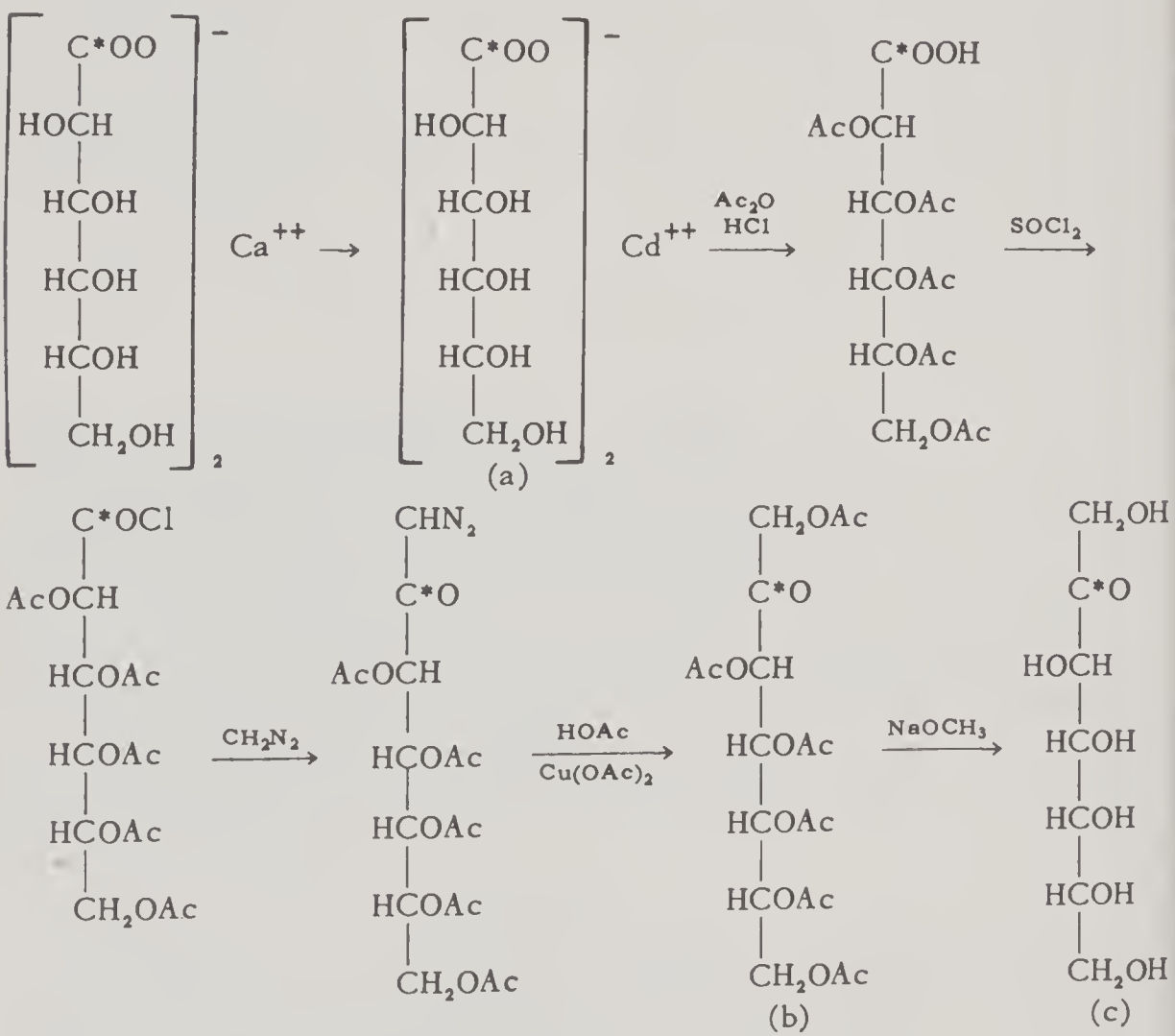
[5]Y. J. Topper and D. Stetten, Jr., J. Biol. Chem., *193*, 149 (1951).

[6]E. Fischer and O. Ruff, Ber., *33*, 2146 (1900).

[7]N. Sperber, H. E. Zaugg and W. M. Sandstrom, J. Am. Chem. Soc., *69*, 915 (1947).

[8]J. C. Sowden and D. J. Kuenne, J. Am. Chem. Soc., *75*, 2788 (1953); H. Kiliani and H. Naegell, Ber., *35*, 3528 (1902).

SEDOHEPTULOSE-2-C^{14}



A. C. Neish and A. C. Blackwood, Can. J. Biochem. Physiol., *33*, 323 (1955).

A. Procedure

(a) *Cadmium D-Altronate-1-C^{14}.* An aqueous solution of calcium D-altronate-1-C^{14} is passed through a column of IR-120 to remove calcium ions. The effluent is neutralized at the boiling point with an excess of cadmium hydroxide, which is then removed by filtration. The filtrate is evaporated to dryness.

(b) *Sedoheptulose-2-C^{14} Hexaacetate.* The synthesis is carried out with 0.49 g. of cadmium D-altronate-1-C^{14} by suitable adaptation of the method of Wolfrom,[1] which follows; yield 0.85 g.

D-Altronic-1-C^{14} Acid Pentaacetate (Note 1). Dry hydrogen chloride is passed slowly through a stirred suspension of 60 g. of cadmium D-altronate and 350 ml. of acetic anhydride at 10°. The temperature rises rapidly after 15–20 minutes and subsides slowly. When the temperature becomes constant, the ice-bath is replaced by a water-bath at 50°, and the stirring and passage of hydrogen chloride are continued for 1 hour. The solution is stored for 12 hours at room temperature and concentrated under reduced pressure at 50–60°. The cooled residue is stirred with 500 ml. of ice and water to hydrolyze the acetic anhydride and dissolve the cadmium salts. The solution is extracted with 3 portions of chloroform, and the combined extract is washed with water, dried over sodium sulfate, decolorized with carbon and evaporated under reduced pressure (Note 2).

D-Altronyl-1-C^{14} Chloride Pentaacetate. A mixture of 25 g. of D-altronic acid pentaacetate, 200 ml. of toluene and 25 g. of purified thionyl chloride is refluxed for 2 hours and evaporated under reduced pressure (Note 2).

1-Deoxy-1-diazosedoheptulose-2-C^{14} Pentaacetate, (1-Deoxy-1-diazo-D-altroketoheptose-2-C^{14} Pentaacetate). To a solution of 6.0 of diazomethane in 200 ml. of absolute ether is added slowly 20 g. of the acid chloride in 100 ml. of absolute ether. The solution is stored at room temperature for several hours and in a refrigerator for 24 hours, then is evaporated (Note 3).

Sedoheptulose-2-C^{14} Hexaacetate, (D-Altroketoheptose-2-C^{14} Hexaacetate) (Note 4). A mixture of 10 g. of 1-deoxy-1-diazosedoheptulose pentaacetate, 150 ml. of glacial acetic acid and 0.01 g. of cupric acetate acid and 0.01 g. of cupric acetate is refluxed for 20 minutes and concentrated by evaporation under reduced pressure.

(c) *Sedoheptulose-2-C^{14}, (D-Altroketoheptose-2-C^{14}).* A mixture of 0.85 g. of the hexaacetate and 5 meq. of sodium methoxide in 20 ml. of methanol is stored overnight and evaporated under reduced pressure. An aqueous solution of the residue is deionized with IR-120, concentrated and chromatographed on Whatman 3 MM paper (Note 5). The ascending chromatogram is developed with 1-butanol-pyridine-water (5:2:1.8). Strips cut from the edge and developed with alkaline silver nitrate[2] (Note 6), or sprayed with orcinol[3] (Note 7), show that the product band is well separated from those of 5 impurities (Note 8). The product band is cut out and eluted with 30% methanol. Evaporation gives a syrup which is dissolved in 2 ml. of water, decolorized with Darco G-60 and evaporated. The over-all radiochemical yield is 12% based on cyanide-C^{14}. A chromatogram developed with 1-butanol-ethanol-water shows only one radioactive

compound, and the synthetic and natural sugars show the same mobility and color with orcinol (Note 9).

B. Notes

1. The procedure is essentially that of Ladenburg[4] for the preparation of fully acetylated aldonic acids.

2. The syrupy product resists crystallization.

3. The syrupy residue could not be crystallized after chromatographic purification.[1]

4. The procedure is similar to that of Wolfrom and Wood.[5]

5. Two 57 × 46-cm. sheets are streaked along the longest dimension.

6. The dry strip is passed rapidly through a silver solution, prepared by diluting 0.1 ml. of saturated aqueous silver nitrate to 20 ml. with acetone and adding water, dropwise with shaking, to redissolve any separated silver nitrate. The dried paper is sprayed with 0.5 *N* alcoholic sodium hydroxide, prepared by diluting saturated sodium hydroxide solution with ethanol. Reducing sugars form dense black spots rather rapidly at room temperature. When reduction is complete, the strip is immersed in 6 *N* ammonium hydroxide to dissolve excess silver oxide, and washed in running water.

7. A solution of 0.5 g. of orcinol, 15 g. of trichloroacetic acid and 100 ml. of 1-butanol (water saturated) is stored in the cold and used within 1 week. The sprayed paper is heated at 105° for 15–20 minutes, sedoheptulose giving a bluish-green color reaction.

8. The two reagents reveal the presence of foreign compounds independently: silver (4), orcinol (1).

9. A further check on the purity of the synthetic sample is made by comparing the ultraviolet absorption spectrum of the orcinol reaction product with that of crystalline sedoheptulosan monohydrate, by the method of Horecker.[6]

C. Other Preparations

In the same manner from cadmium D-altronate-2-C^{14} is prepared sedoheptulose-3-C^{14}; over-all radiochemical yield 0.8% based on cyanide-C^{14}.

[1]M. L. Wolfrom, J. M. Berkebile and A. Thompson, J. Am. Chem. Soc., *74*, 2197 (1952).

[2]W. E. Trevelyan, D. P. Proctor and J. S. Harrison, Nature, *166*, 444 (1950).

[3]R. Klevstrand and A. Nordal, Acta Chem. Scand., *4*, 1320 (1950).

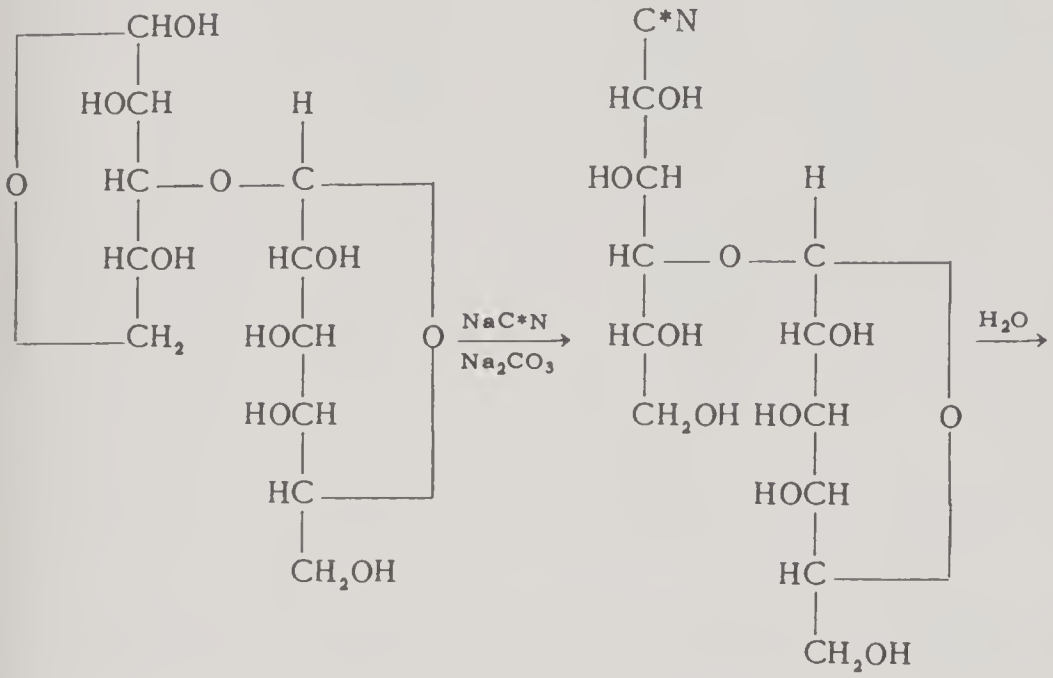
[4]K. Ladenburg, M. Tishler, J. W. Wellman and R. D. Babson, J. Am. Chem. Soc., *66*, 1217 (1944).

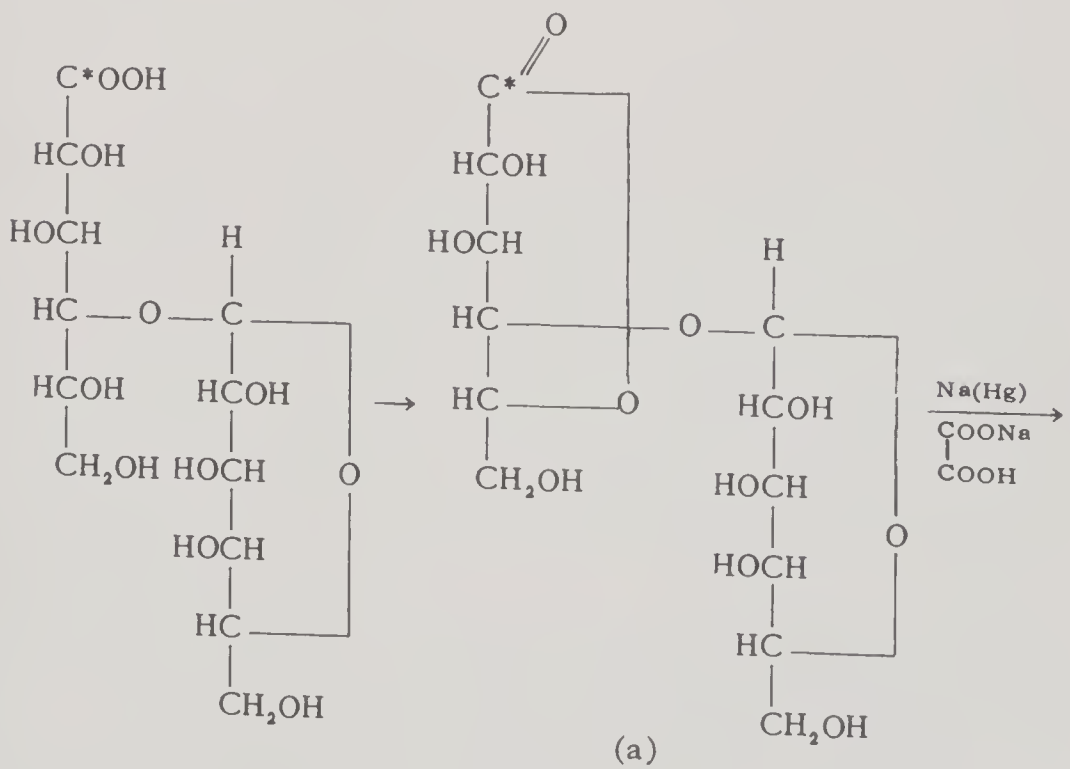
[5]M. L. Wolfrom and H. B. Wood, J. Am. Chem. Soc., *73*, 730 (1951).

[6]B. L. Horecker, P. Z. Smyrniotis and H. Klenow, J. Biol. Chem., *205*, 661 (1953).

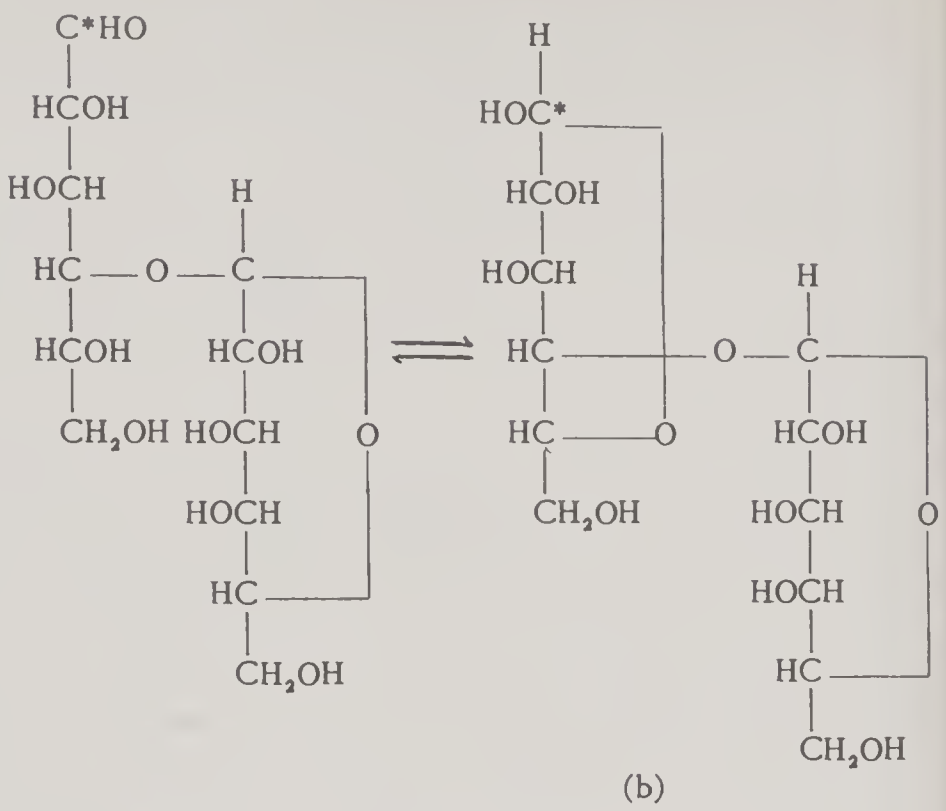
LACTOSE-1-C^{14}

(4-*O*-β-D-Galactopyranosyl-D-glucopyranose-1-C^{14})





(a)



(b)

H. L. Frush and H. S. Isbell, J. Research Nat. Bur. Standards, *50*, 133 (1953).

A. Procedure

(a) *Lactobiono-δ-lactone-1-C*^{14}. To a mixture of 0.343 g. (1 mmole) of 3-*O*-β-D-galactopyranosyl-D-arabinose (Note 1) and 0.084 g. of sodium bicarbonate at 0° is added 5 ml. of a solution containing 1 mmole of sodium hydroxide and 1 mmole of sodium cyanide-C^{14} (Note 2). The tube is stoppered and stored for 3 days at room temperature; then the mixture of cyanohydrins is hydrolyzed by heating for 6 hours at 70° in an air stream (Note 3). The residue is dissolved in 50 ml. of water and passed through a cation exchange column of Amberlite IR-100. The combined effluent and washings are concentrated to 2 ml. under reduced pressure, and the solution of lactobionic-1-C^{14} acid (Note 4) is diluted with an equal volume of methyl cellosolve, seeded and lactonized by the slow evaporation of solvent at room temperature in a stream of air. The semi-solid mass is stored for 1 week over calcium chloride, with occasional addition of methyl cellosolve to maintain a moist condition; then the storage is continued for at least another week without addition of solvent. The residue is dissolved in 50 parts of methyl cellosolve at 90–100° and filtered, and the cooled filtrate is seeded and concentrated under reduced pressure to crystallize the product. The mother liquor is removed by capillary pipette, and the crystals are washed with cold solvent; yield 0.0525 g. Four portions of carrier, totalling 1.4 g., are crystallized from the mother liquor with a recovery of 1.253 g., making the total radiochemical yield 54.2%.

(b) *Lactose-1-C^{14}* (Note 5). To a stirred mixture of 0.20 g. of sodium oxalate, 0.18 g. of oxalic acid, 0.5 mmole of lactobiono-δ-lactone-1-C^{14} and 10 ml. of water is added 1,2 g. of 5 per cent sodium amalgam pellets. The mixture is stirred vigorously with ice-bath cooling, until the amalgam is spent. The mercury is removed by pipette, and the solution is made just basic to phenolphthalein with sodium hydroxide solution, then just acidic by dropwise addition of oxalic acid solution. The solution is diluted with five volumes of methanol, and the precipitated salts are filtered off and washed with methanol. The combined filtrate is evaporated under reduced pressure, the residual syrup is dissolved in 4 volumes of methanol, and the precipitated salts are removed by filtration without delay. The filtrate is re-evaporated, and the residue is dissolved in water and passed through a deionizing column (Note 6) with thorough washing. The combined solution is concentrated under reduced pressure and lyophilized. The residue is dissolved in a few drops of water, and the solution is seeded and treated with methanol to incipient turbidity and then with isopropyl alcohol as crystallization progresses. After storage for several days in a refrigerator, the mother liquor is removed and the product is washed with methanol - 2-propanol (2:1). Three portions of carrier are crystallized from the mother liquor, making the radiochemical yield 68.4% (Note 7).

B. Notes

1. Directions are given by Frush and Isbell for preparing the starting material from calcium lactobionate[1] by oxidative degradation.

2. This condensation has been studied under various experimental conditions by the tracer technique, in order to direct the reaction to the formation of the desired epimer. Following hydrolysis of the cyanohydrins, the tagged lactobionic acid was isolated as the calcium lactobionate-calcium bromide double salt for assay by isotopic dilution. Both sodium carbonate and calcium chloride favor formation of the 4-galactosyl-gluconic epimer, while the more acidic sodium bicarbonate favors the 4-galactosyl-mannonic epimer.

3. Water is added as necessary.

4. Sodium lactobionate-1-C^{14} is prepared by neutralizing the acid solution with sodium hydroxide solution to the phenolphthalein end point. The solution is decolorized with charcoal, filtered and evaporated. The residue is treated with methanol to the point of incipient turbidity, seeded and set aside for several days. The salt[2] is recrystallized easily from water-methanol. The chemical yield is 40%, and the radiochemical yield (with the use of carrier) is 50%.

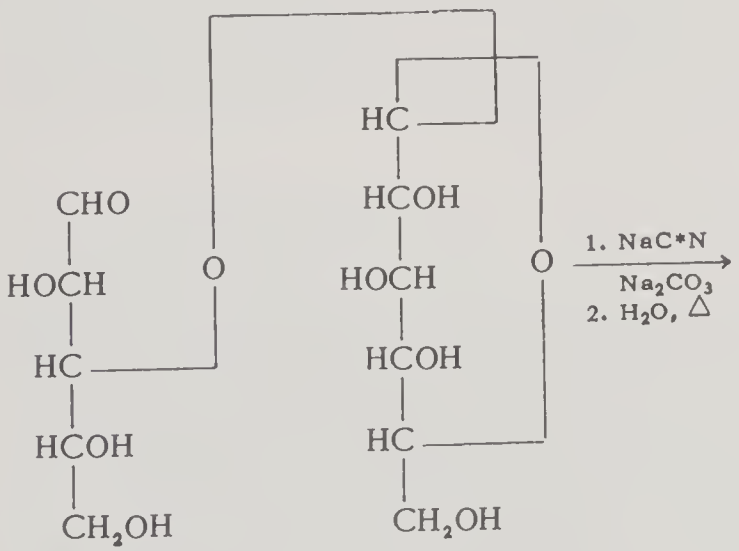
5. The reduction is performed in the manner described for the preparation of D-glucose-1-C^{14}.

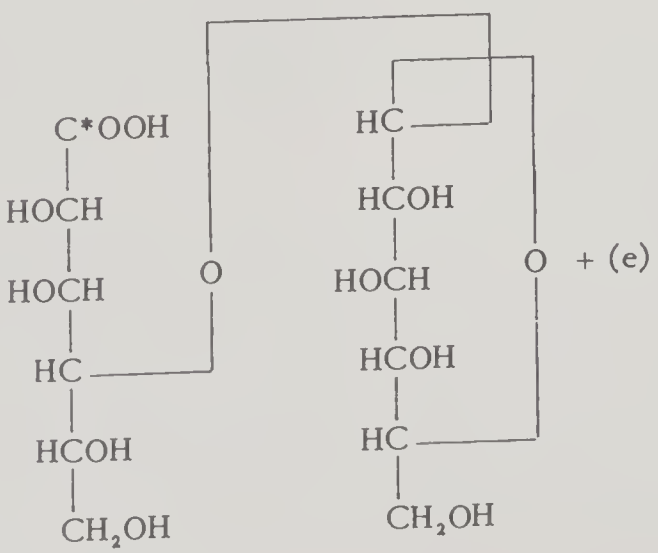
6. Amberlite IR-100 and Duolite A-4, 10 ml. each.

7. The product may be prepared in 38% yield from 2 mmoles of sodium cyanide-C^{14}, without isolation of intermediates, but the higher proportion of isotopic contaminants in the crude product hardly recommends it. Radioautographic and chromatographic[3] analysis of lactose-1-C^{14} prepared in this manner has revealed the presence[4] of seven compounds, including three radioactive ones. Purification is accomplished[4] by column chromatography on charcoal-Celite.[5]

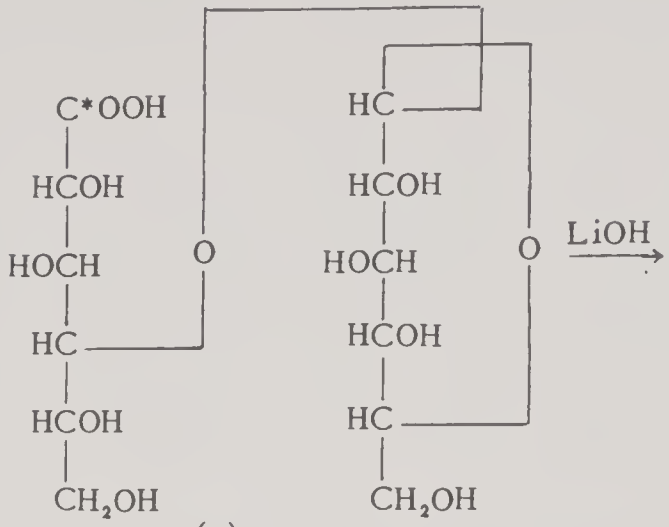
[1]H. S. Isbell and H. L. Frush, J. Research Nat. Bur. Standards, *6*, 1145 (1931).
[2]G. E. N. Nelson and F. H. Stodola, J. Am. Chem. Soc., *75*, 1748 (1953).
[3]E. F. McFarren, K. Brand and H. R. Rutkowski, Anal. Chem., *23*, 1146 (1951).
[4]F. J. Carleton, S. Misler and H. R. Roberts, J. Biol. Chem., *214*, 427 (1955).
[5]R. L. Whistler and D. F. Durso, J. Am. Chem. Soc., *72*, 677 (1950).

MALTOSE-1-C^{14}

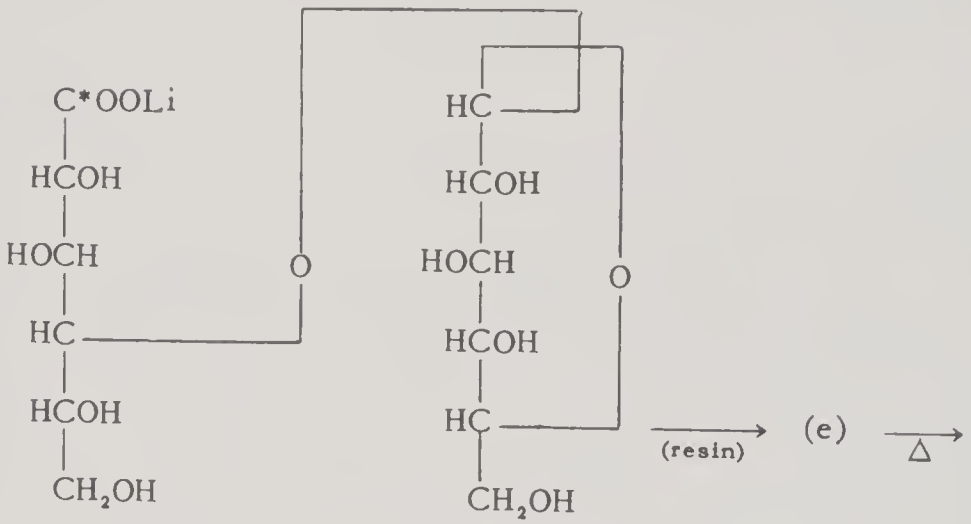




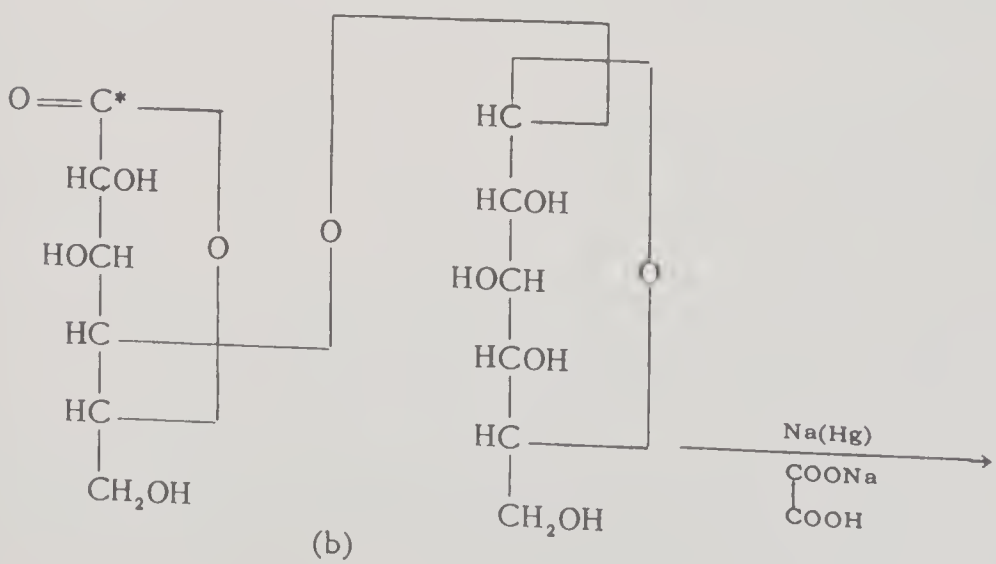
(d)


C*OOH
HCOH
HOCH
HC
HCOH
CH2OH
O
HC
HCOH
HOCH
HCOH
HC
CH2OH
O
LiOH

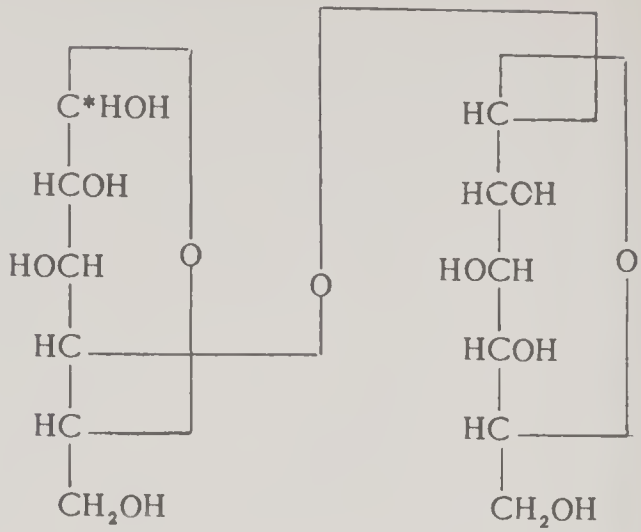
(e)


C*OOLi
HCOH
HOCH
HC
HCOH
CH2OH
O
HC
HCOH
HOCH
HCOH
HC
CH2OH
O
(resin)
(e)
Δ

(a)


O=C*
HCOH
HOCH
HC
HC
CH2OH
O
O
HC
HCOH
HOCH
HCOH
HC
CH2OH
O
Na(Hg)
COONa
COOH

(b)



(c)

H. S. Isbell and R. Schaffer, Atomic Energy Commission Report, NBS-4031; Nuc. Sci. Abstracts, 9, 6628 (1955).

A. Procedure

(a) *Lithium Maltobionate-1-C^{14} Trihydrate.* To an ice-cold solution of 8.85 mmoles of sodium cyanide-C^{14} and 9.6 mmoles of sodium carbonate (Note 1) in 25 ml. of water is added a cold solution of 2.81 g. (9.0 mmoles) of 3-*O*-α-D-glucosyl-D-arabinose (Note 2) in 65 ml. of water. The mixture is stirred at 8° for 3 days, and at room temperature for 1 day, then is treated with 0.53 g. of sodium carbonate and heated at 80° until no further ammonia is evolved. The dark solution is passed through a column containing 220 ml. of ice-cold Amberlite IR-100H, by washing with 1 l. of ice water. The effluent is swirled for a few minutes under reduced pressure (Note 3), then is neutralized with lithium hydroxide and concentrated to 25 ml. under reduced pressure. The solution is diluted with 75 ml. of methanol and filtered. The filtrate is concentrated to 5 ml. under reduced pressure, then treated with a few drops of 2-propanol and seeded (Note 4), more 2-propanol being added as crystallization proceeds. The product is collected, washed and recrystallized from aqueous 2-propanol; yield 0.779 g. (Note 5). The radiochemical yield (21.0%) is raised to 53.7% by the use of 3.0 g. of carrier (Note 6).

(b) *Maltobiono-δ-lactone-1-C^{14}.* An aqueous solution of 0.212 g. of lithium maltobionate-1-C^{14} trihydrate is passed through a column containing 7 ml. of ice-cold Amberlite IR-120H, by washing with 100 ml. of ice water. The effluent is lyophilized, and the residue is dissolved in methyl cellosolve and transferred into a reduction tube (Note 7). The solution is seeded, warmed to 90° for a few minutes and kept at 50° under a gentle air stream. Whenever the crystallizing solution becomes concentrated during 10 days, 0.5 ml. of the solvent is added, the tube is reheated at 90°, and the contents are reconcentrated at 50°. The residue is dried under vacuum over calcium chloride.

(c) *Maltose-1-C^{14} Hydrate*, (*4-O-α-D-Glucosyl-D-glucose-1-C^{14}*). The reduction tube is cooled with an ice-bath and fitted with a stirrer; then through the side-arm is added in rapid succession: 2.6 g. of sodium hydrogen oxalate, 20 ml. of ice water and 4.0 g. of 3.7% sodium amalgam pellets (Note 7). The mixture is stirred for 2.5 hours, and the aqueous solution is separated, neutralized with dilute sodium hydroxide and diluted with 3 volumes of methanol to precipitate the inorganic salts. The filtered solution is concentrated to a syrup, which is dissolved in 25 ml. of ice water and passed in turn through ice-cold columns containing 25 ml. of Amberlite IR-120H and 25 ml. of Duolite A-4. The solution is washed through the columns with 250 ml. of ice-water (Note 7), and the effluent is lyophilized (Note 8). The residue is dissolved in methanol, and the filtered solution is evaporated. An aqueous solution of the residue is passed through a filter coated with decolorizing carbon, then is concentrated to 1 ml., seeded and treated with 2-propanol. The product is collected, washed and recrystallized from aqueous 2-propanol; yield 0.098 g. The radiochemical yield is raised from 54% to 77% by the use of carrier (Note 9).

B. Notes

1. The maximum yield of the desired gluconic epimer (maltobionic nitrile) is obtained in the presence of sodium carbonate.

2. The starting material is prepared by the procedure of Isbell.[1]

3. Carbon dioxide is removed under reduced pressure.

4. Directions are given by Isbell for preparing lithium maltobionate trihydrate by electrolysis of β-maltose; $[\alpha]_D^{20}$ + 97.3° (*c* 8.7, water).

5. The product is dried overnight at 20 mm. over calcium chloride, m.p. 106.5–108°. Three molecules of water of crystallization are lost at 105° under vacuum (2 hours).

6. The mother liquors contain lithium 4-*O*-α-D-glucosyl-D-mannonate-1-C^{14}, (d).

7. See D-mannose-1-C^{14}.

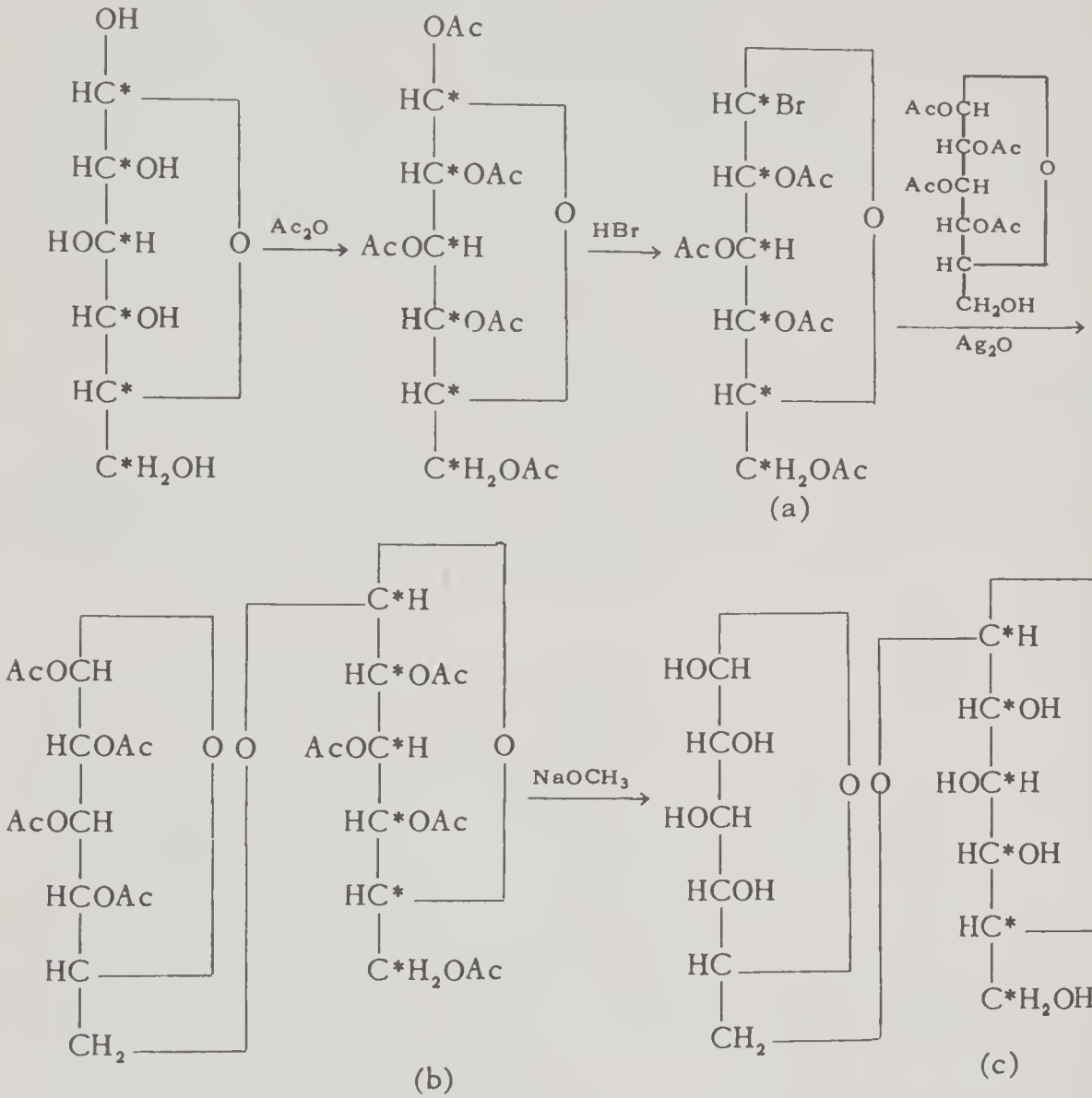
8. Unreacted maltobionic-1-C^{14} acid may be recovered as the lithium salt (17%) by washing the Duolite A-4 with 300 ml. of 10% acetic acid, lyophilizing the effluent and neutralizing an aqueous solution with lithium hydroxide.

9. Trial experiments have shown that the reduction of 0.5 mmole of maltobiono-δ-lactone in 20 ml. of water with 2.3 g. of 5% amalgam in the presence of 1.6 g. of sodium hydrogen oxalate gives 94% yields of maltose, as determined by the Scales method.[2]

[1]H. S. Isbell and J. D. Moyer, Abstracts Amer. Chem. Soc. Meeting, New York, Sept. 12, 1954, p. 24D; G. Zemplén, Ber., 59, 2408 (1926).

[2]F. J. Bates and associates, *Polarimetry, saccharimetry and the sugars*, NBS Circular 440, p. 189 (1942).

6-*O*-β-D-GLUCOPYRANOSYL-C_6^{14}-D-GLUCOSE
(C_6^{14}-β-Gentiobiose)



J. C. Sowden and A. S. Spriggs, J. Am. Chem. Soc., 76, 3539 (1954).

A. Procedure

(a) *α-D-Glucosyl-C_6^{14} Bromide Tetraacetate, Acetobromoglucose-C_6^{14}*). D-Glucose-C_6^{14} (0.950 g.) is converted in 1.13-g yield, presumably by the Redemann and Niemann[1] (Note 1).

(b) *6-O-β-D-Glucopyranosyl-C_6^{14}-D-glucose Octaacetate, (C_6^{14}-β-Gentiobiose Octaacetate*). The glucosyl bromide (1.13 g.) is condensed with 0.965 g. of β-D-glucopyranose 1,2,3,4-tetraacetate (Note 2) by the procedure of Reynolds,[2] which follows; yield 0.821 g., m.p. 195°, $[\alpha]_D^{25}$ −5.1° (c 2 in $CHCl_3$).

A mixture of 35 g. of β-D-glucose 1,2,3,4-tetraacetate, 25 g. of silver oxide (Note 3), 100 g. of Drierite and 100 ml. of alcohol-free chloroform (Note 4) is stirred for 1 hour in a flask protected from light and moisture.

The mixture is treated with 5 g. of iodine; then a solution of 41.3 g. (1 equivalent) of acetobromoglucose in 150 ml. of dry, alcohol-free chloroform is added during 1 hour. The mixture is stirred for 24 hours, then filtered through "Filter-Cel," the residue being washed with chloroform. The filtrate is evaporated under reduced pressure, and the product is crystallized from 2 liters of hot absolute ethanol; yield 50.5 g. (74.1%), m.p. 195–196° (cor.).

(c) *6-O-β-D-Glucopyranosyl-C_6^{14}-D-glucose.* The C_6^{14}-β-gentiobiose octaacetate (0.200 g.) is deacetylated according to the procedure of Thompson,[3] which follows. The yield of anhydrous product, dried under vacuum at 110° over phosphorus pentoxide, is 0.052 g., m.p. 193–194° (Note 5).

A mixture of 15 g. of β-gentiobiose octaacetate and 180 ml. of 0.05 *N* sodium methoxide in dry methanol is shaken for 1 hour, then diluted with sufficient water to form a solution which is passed through a deionizing column of Amberlite IR-120 and Duolite A-4. The effluent is evaporated under reduced pressure, and the residue is dried by repeated evaporation of added absolute ethanol. A filtered solution of the syrup in 40 ml. of hot methyl cellosolve is seeded and stored overnight at 30° (Note 6). The crystalline product is collected and washed with absolute ethanol; yield 6.7 g. (89%), m.p. 187–189° (cor.), $[\alpha]_D^{28}$ −1.5° (initial, extrapolated) → +10.6° (final, *c* 4, water) (Note 7).

B. Notes

1. The uniformly labeled D-glucose is prepared by biosynthetic means; see D-sorbitol-C_6^{14}, Note 1.

2. The preparation of β-D-glucose 1,2,3,4-tetraacetate (m.p. 128–129°) is described by Reynolds,[2,4] and Lardy.[5] The α-isomer is ether-soluble, the β-isomer is insoluble.

3. Silver oxide is prepared[6] by adding a hot solution of 0.23 parts of sodium hydroxide in 10 parts of water to a solution of 1 part of silver nitrate in 10 parts of water on a steam-bath. The precipitate is washed by decantation with hot water, then with alcohol, and dried in a vacuum desiccator over phosphorus pentoxide.

4. The Drierite is preheated to 240° for 2 hours. U.S.P. chloroform is shaken for 1 hour with 12% sulfuric acid, separated, neutralized with sodium bicarbonate, washed with water, dried over calcium chloride, distilled, dried over phosphorus pentoxide, redistilled, and stored over Drierite.

5. The chromatography of β-gentiobiose on columns of Darco G-60-Celite No. 535, and elution with water (5% alcohol and 15% alcohol) are described by Sowden. The use of the isotopic product in radioisotopic dilution analysis of a complex sugar mixture, with the advantage of non-

reliance upon the efficiency of isolating the component being determined, is described by Sowden.

6. The crystalline phase forms more readily at elevated temperatures.

7. Recrystallization from methyl cellosolve gives a 95% recovery, m.p. 190° (cor.).

[1]*Organic Syntheses*, Vol. 22, Wiley, New York, 1942, p. 1.

[2]D. D. Reynolds and W. L. Evans, J. Am. Chem. Soc., *60*, 2559 (1938); B. Helferich and W. Klein, Ann., *450*, 219 (1926).

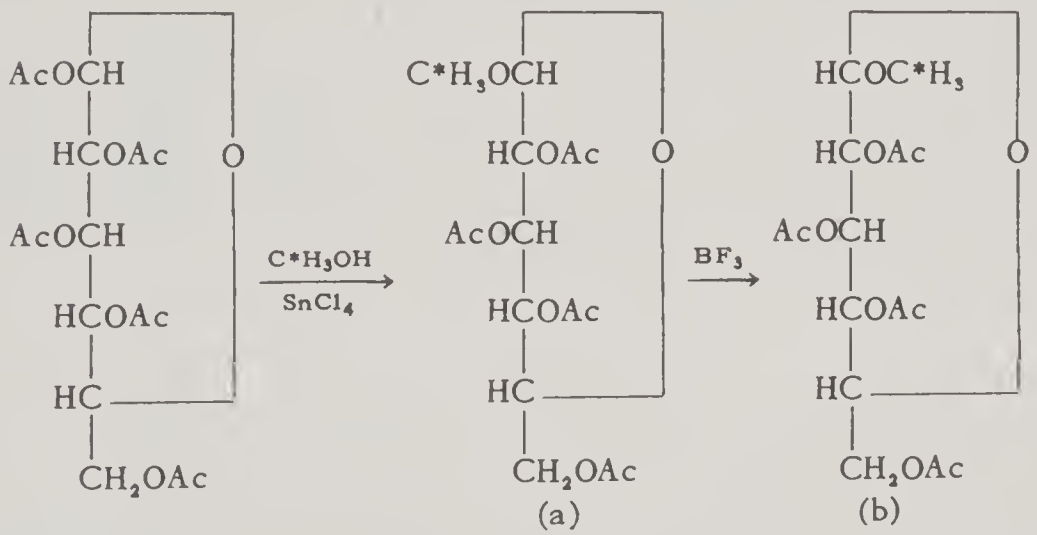
[3]A. Thompson and M. L. Wolfrom, J. Am. Chem. Soc., *75*, 3605 (1953).

[4]*Organic Syntheses*, Vol. 22, Wiley, New York, 1942, p. 56.

[5]H. A. Lardy and H. O. L. Fischer, J. Biol. Chem., *164*, 513 (1946).

[6]B. Helferich, E. Bohn and S. Winkler, Ber., *63*, 989 (1930).

α-METHYL-C^{14}-D-GLUCOPYRANOSIDE TETRAACETATE



R. U. Lemieux and W. P. Shyluk, Can. J. Chem., *33*, 120 (1955).

A. Procedure (Note 1)

(a) *β-Methyl-C^{14}-D-Glucopyranoside Tetraacetate* (Note 2). To a mixture of 1.8 mmoles of freshly distilled stannic chloride, 1.77 mmoles of methanol-C^{14} and 9 ml. of dry benzene is added 1.77 mmoles of β-D-glucopyranose pentaacetate. The solution is heated at 40° for 1 hour, washed with cold water, bicarbonate and water, then dried over sodium sulfate and evaporated under vacuum. The residue is triturated with methanol and stored at −10° for 7 hours. The product is collected and recrystallized three times from methanol, m.p. 104.5–105° (Note 3).

(b) *α-Methyl-C^{14}-D-glucopyranoside Tetraacetate.* A mixture of 0.0114 g. of β-methyl-C^{14}-D-glucopyranoside tetraacetate (and 0.0998 g. of methyl β-L-glucopyranoside tetraacetate) and 2 ml. of absolute chloroform (Note 4) is saturated with boron trifluoride (Note 5) and stored at room temperature for 24 hours. The solution is washed with sodium bicarbonate (Note 6), dried and evaporated. Isotope dilution analysis (Note 7) indicates that the yield is 92%. Purification is accomplished by recrystallization from ethanol, m.p. 101.5–102.5° (Note 8).

B. Notes

1. The preparations are incidental to a study of the intramolecular mechanism of anomerization of acetylated alkylglucopyranosides; see Pacsu.[1] It is postulated that the reaction is best rationalized as the result of an unsuccessful attempt by the acidic environment to bring about glycosidic cleavage and that it proceeds by way of an ion-pair intermediate in which the anion is derived from the aglycon group. The procedure is an adaptation of that of Lemieux.[2]

2. The method is that of Lindberg.[3]

3. The yield is 53% at the 25-mmole scale after one recrystallization m.p. 104–105°, $[\alpha]_D^{25}$ −17° (*c*, 2.8 in chloroform);[2] α-D-glucosyl chloride tetraacetate is a by-product of the synthesis.

4. The solvent is prepared immediately before use: reagent grade chloroform is washed with water, dried over calcium chloride, refluxed over phosphorus pentoxide and distilled.

5. Directions are also given by Lemieux for effecting the anomerization with titanium tetrachloride (76% yield).

6. Lindberg[3] reports that a slight yellow gelatinous precipitate disappears, both phases becoming colorless.

7. An aliquot of the syrupy product (0.0083 g.) was diluted with 0.101 g. of carrier and recrystallized six times from ethanol to constant specific activity (92% of theory, based on an intramolecular mechanism and quantitative yield).

Another aliquot (0.0078 g.) was diluted with 0.0995 g. of pure α-methyl-L-glucopyranoside tetraacetate (preparation given) and recrystallized seven times from ethanol, m.p. 102–102.5°, specific activity 0, $[\alpha]_D^{25}$ −130.5° (*c*, 1 in chloroform).

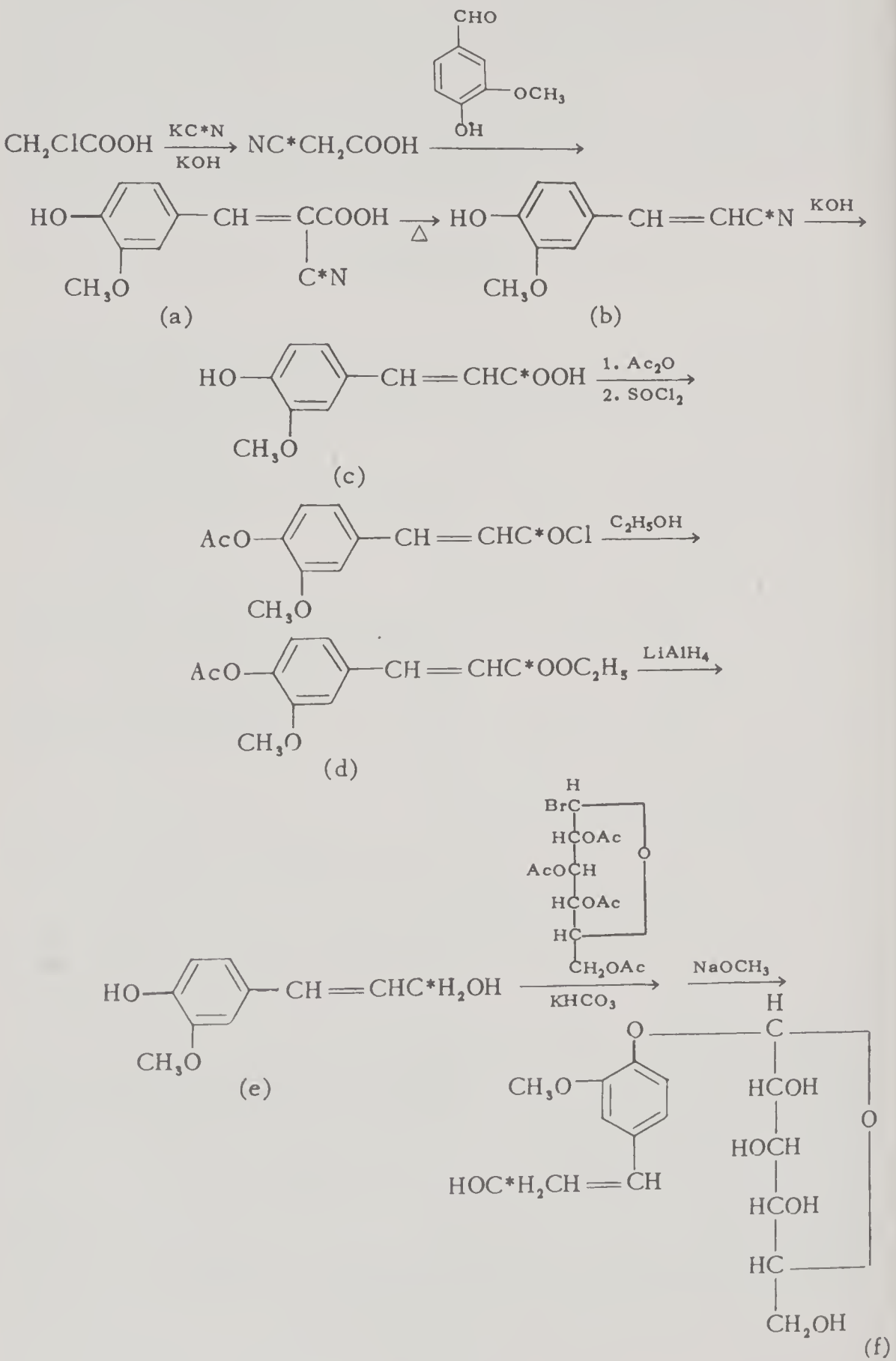
8. α-Methyl-D-glucopyranoside tetraacetate, $[\alpha]_D^{20}$ +131°.[3]

[1] E. Pacsu, Ber., *61*, 137, 1508 (1928); E. Pacsu, J. Am. Chem. Soc., *52*, 2563, 2568, 2571 (1930); B. Lindberg, Acta Chem. Scand., *3*, 1153 (1949).

[2] R. U. Lemieux and W. P. Shyluk, Can. J. Chem., *31*, 528 (1953).

[3] B. Lindberg, Acta Chem. Scand., *2*, 426, 534 (1948).

3-(4-β-D-GLUCOSYLOXY-3-METHOXYPHENYL)-2-PROPEN-1-OL-1-C^{14} (C_1^{14}-Coniferin)



K. Freudenberg and F. Bittner, Ber., 86, 155 (1953).

A. Procedure

(a) *α-Cyano-C^{14}-ferulic Acid,* (*α-Cyano-C^{14}-4-hydroxy-3-methoxycinnamic Acid*) (Note 1). To a frozen solution of 0.022 g. of potassium cyanide-C^{14} and 0.036 g. of potassium hydroxide in 0.3 ml. of water is added 0.058 g. of chloroacetic acid. The mixture is stored at 25° for 3 hours in a sealed tube, then treated with 0.027 g. of potassium cyanide and heated at 80° for 1 hour. To the mixture is added a solution of 0.150 g. of vanillin in 1.5 ml. of 10% sodium hydroxide. After standing for 3 hours at room temperature, the mixture is heated at 60° for 30 minutes, cooled and acidified with 0.5 ml. of concentrated hydrochloric acid, and the product is filtered off. The filtrate is made alkaline, treated with 0.032 g. of carrier and again acidified to crystallize a second crop. The combined crude product is dissolved in methanol, and the solution is evaporated to dryness in a centrifuge tube. The residue is extracted with several portions of hot benzene, chiefly to remove vanillin; yield 65–70%.

(b) *Ferulonitrile-C^{14}* (Note 2). A mixture of the α-cyano-C^{14}-ferulic acid, 3 ml. of cyclohexanol and 1 ml. of *N,N*-dimethylaniline is refluxed for 2 hours at 170° (Note 3), then evaporated at 50° (1 μ). The residue is dissolved in 20 ml. of chloroform, and the solution is passed through a column of aluminum oxide (4 × 20 mm.) and evaporated to dryness (Note 4)

(c) *Ferulic-C^{14} Acid,* (*4-Hydroxy-3-methoxycinnamic-C^{14} Acid*). The crude nitrile is refluxed for 4.5 hours with 4 ml. of 20% potassium hydroxide under nitrogen. The cooled solution is acidified with concentrated hydrochloric acid, and the product is extracted with a total of 50 ml. of ethyl acetate (Note 5).

(d) *Ethyl O-Acetylferulate-C^{14}*. The ferulic-C^{14} acid is converted in turn to: *O*-acetylferulic-C^{14} acid, *O*-acetylferuloyl-C^{14} chloride and ethyl *O*-acetylferulate-C^{14}; yield 33% based on potassium cyanide (Note 6).

A suspension of ferulic acid in acetic anhydride is heated for 1.5 hours, then the excess reagent is hydrolyzed with warm water and the *O*-acetylferulic acid is crystallized (Note 7). Crude *O*-acetylferulic acid is refluxed for 1 hour with an excess of purified thionyl chloride containing a drop of pyridine. The solution is evaporated to dryness, the crude *O*-acetylferuloyl chloride is treated with benzene, and the evaporation if repated to remove traces of thionyl chloride (Note 8). Esterification is accomplished by refluxing the acid chloride with an excess of absolute ethanol and evaporating the solution (Note 9).

(e) *3-(4-Hydroxy-3-methoxyphenyl)-2-propen-1-ol-1-C^{14}*, (*C$_1^{14}$-Coniferyl Alcohol*). The C^{14}-ester is reduced with 0.032 g. of lithium aluminum hydride during 8 hours according to the procedure of Allen,[1] which follows.

To a stirred solution of 2.5 g. (65.8 mmoles) of lithium aluminum hydride in 400 ml. of absolute ether, cooled to −15° in an anhydrous sys-

tem under nitrogen, is added a solution of 9 g. (34 mmoles) of ethyl *O*-acetylferulate in 700 ml. of ether during 2 hours. The mixture is stirred for 2 hours, then stored overnight and allowed to come to room temperature. The yellow salt containing lithium and aluminum is collected and washed with two 100-ml. portions of absolute ether to remove reagent and unreacted ester; yield 9 g. of salt (Note 10).

The C^{14}-salt is decomposed in the cold with dilute hyposulfite solution, and the solvent is removed under high vacuum.

(f) *3-(4-β-D-Glucosyloxy-3-methoxyphenyl)-2-propen-1-ol-1-C^{14}*, (*C_1^{14}-Coniferin*). A mixture of the crude C_1^{14}-coniferyl alcohol, 0.070 g. of potassium bicarbonate, 2 ml. of water, 0.146 g. of D-acetobromoglucose (Note 11) and 2 ml. of acetone is shaken at room temperature for 36 hours, then evaporated to dryness under high vacuum. The residue is dissolved in methylene chloride, and the solution is washed with 0.1 *N* sodium hydroxide until no yellow color is extracted (Note 12), then is evaporated (Note 13).

The residue (C_1^{14}-coniferin tetraacetate) is hydrolyzed by the method of Zemplén;[2] yield 0.019 g. In general, this consists of treating acetylated sugars with methanol containing considerably less than the calculated amount of sodium methozide at room temperature, or on a steam-bath. The mixture is saturated with carbon dioxide, then diluted with hot dioxane, and the carbonate is separated by centrifuging. The solution is concentrated to 0.5 ml., diluted with 4 ml. of benzene and stored for 24 hours in a refrigerator. The product is separated by centrifuging and recrystallized from 0.1 ml. of water; yield 0.019 g. (Note 14). The radiochemical yield is raised by crystallizing two 7-mg. portions of carrier from the mother liquor.

B. Notes

1. The procedure is a modification of the method of Lapworth.[3]
2. The procedure is a modification of the method of Freudenberg.[4]
3. Copper powder is used to facilitate boiling.
4. Ferulonitrile crystallizes in the form of yellow crystals, m.p. 112°; acetyl derivative, m.p. 102–103°.
5. Ferulic acid crystallized from water melts at 168–169°.
6. The method is after that of Freudenberg.[5] Also see Other Preparations.
7. *O*-Acetylferulic acid crystallized from dilute acetic acid melts at 194–196°.
8. *O*-Acetylferuloyl chloride recrystallized[6] from hot benzene melts at 133–134°; yield 82%.

9. Ethyl *O*-acetylferulate,[1] m.p. 120–121°, b.p. 186–189° (5 mm.); yield 70%.

10. The lithium aluminum salt is decomposed[1] to a lithium salt in the presence of water. The free alcohol may be isolated by decomposing the mixed salt with ammonium carbonate solution and by ether extraction; yield 42.6%, m.p. 72–73°. Coniferyl alcohol crystallizes with reluctance and slowly polymerizes on standing.

11. A preparation of acetobromoglucose (m.p. 88–89°) is described by Redemann and Niemann.[7] Potassium carbonate is used by Freudenberg and Fuchs.[8]

12. The alkaline extract and the methylene chloride-insoluble residue contains unreacted C_1^{14}-coniferyl alcohol which is recovered with the use of carrier.

13. Coniferin tetraacetate[9] melts at 132°.

14. Coniferin dihydrate becomes anhydrous after 4 hours at 100°, m.p. 186°. The solubility is 1 g. in 200 ml. of water. The ultraviolet absorption spectrum is described by Patterson.[10] The paper chromatography of coniferin with 7 solvents is described by Janot.[11]

Directions are given by Freudenberg and Bittner for converting the product to a dehydrogenation C^{14}-polymer (DHP)[8] by treatment with mushroom dehydrogenase at pH 6.5; yield 16.5 g. from 18.8 g. of coniferyl alcohol. A degradation of the polymer is described.

C. Other Preparations

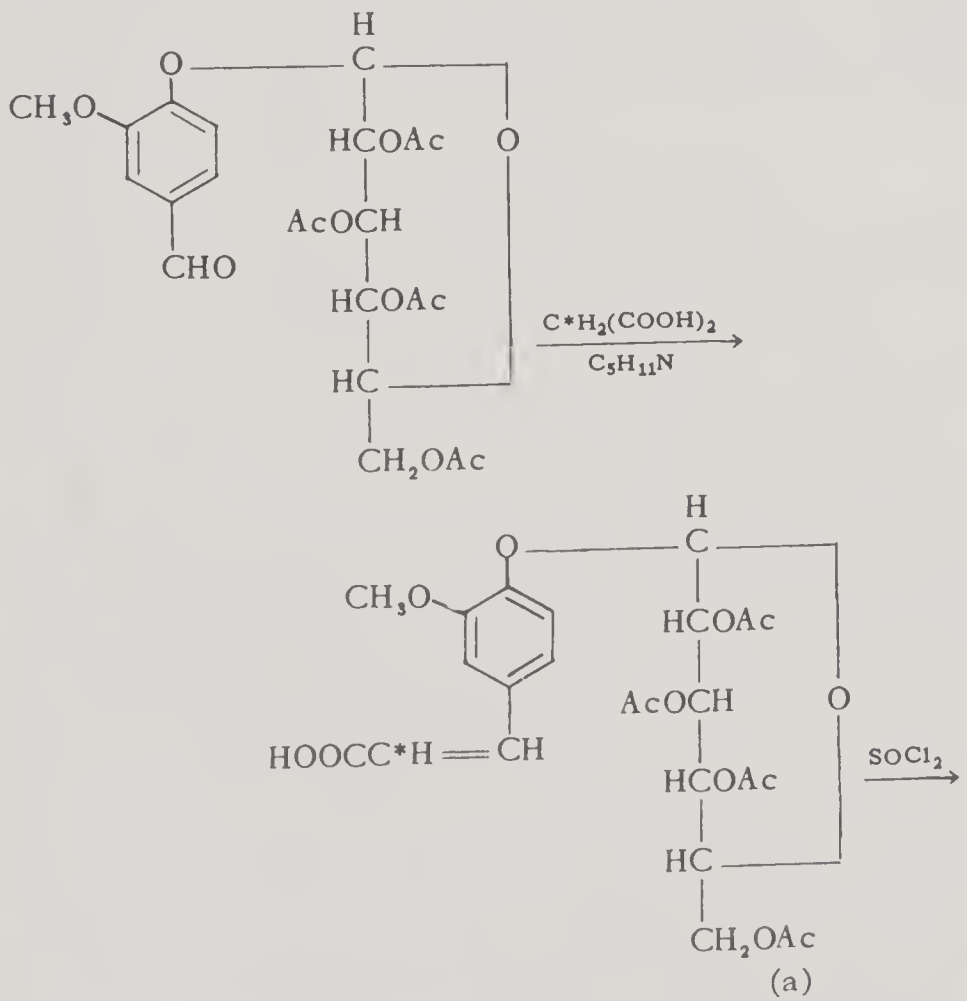
A similar preparation of 3-(4-β-L[and D]-glucosyloxy-3-methoxyphenyl)-2-propen-1-ol-1-C^{14}, employing L[and D]-acetobromoglucose,[12] has been described in some detail by Freudenberg and Fuchs.[8] Ethyl *O*-acetylferulate-C^{14} was formed by esterifying *O*-acetylferulic-C^{14} acid in cold trifluoroacetic anhydride solution with an excess of ethanol; see ethyl 3,4-dimethoxycinnamate-C^{14}.

The preparation of 3-(3,4-dimethoxyphenyl)-2-propen-1-ol-1-C^{14} has been described by Freudenberg.[8] A solution of ammonium malonate-1-C^{14} (prepared from calcium malonate-1-C^{14} with the calculated amount of hydrochloric acid and a slight excess of ammonium oxalate) in pyridine is treated with veratraldehyde in the presence of a trace of piperidine and aniline. The mixture is heated at 50° for 24 hours and at 100° for 1 hour, the evolved carbon-C^{14} dioxide being recovered. The mixture is acidified, and the 3,4-dimethoxycinnamic-C^{14} acid is purified by reprecipitation; yield 60%, m.p. 180–181°. To a frozen solution of the acid in trifluoroacetic anhydride (Dry Ice-bath) is added an excess of ethanol; then the shaken mixture is warmed to room temperature and evaporated under vacuum. The crude ethyl 3,4-dimethoxycinnamate-C^{14} is vacuum-

sublimed at 80–100°; yield 90%, m.p. 61°. The product obtained by reduction of the ester is purified by vacuum sublimation at 70–80°; yield 80%, m.p. 79°.

[1]C. F. H. Allen, Jr. and J. R. Byers, Jr., J. Am. Chem. Soc., *71*, 2683 (1949).
[2]G. Zemplén, A. Gerecs and I. Hadacsy, Ber., *69B*, 1827 (1926).
[3]A. Lapworth and J. A. McRae, J. Chem. Soc., *121*, 1699 (1922).
[4]K. Freudenberg and R. Dillenberg, Ber., *84*, 67 (1951).
[5]K. Freudenberg and H. H. Hübner, Ber., *85*, 1181 (1952).
[6]L. S. Fosdick and A. C. Starke, Jr., J. Am. Chem. Soc., *62*, 3352 (1940).
[7]*Organic Syntheses*, Vol. 22, Wiley, New York, 1942, p. 1.
[8]K. Freudenberg and W. Fuchs, Ber., *87*, 1824 (1954).
[9]H. Pauly and K. Feuerstein, Ber., *60*, 1031 (1927).
[10]R. F. Patterson and H. Hibbert, J. Am. Chem. Soc., *65*, 1862 (1943).
[11]M. Janot, E. Saias and M. Foucher, Bull. soc. chim. biol., *35*, 1101 (1953).
[12]K. Freudenberg and F. Bittner, Ber., *83*, 603 (1950); M. Barczai-Martos and F. Korösy, Nature, *165*, 369 (1950); J. C. Sowden and H. O. L. Fischer, J. Am. Chem. Soc., *69*, 1963 (1947).

3-(4-β-D-GLUCOSYLOXY-3-METHOXYPHENYL)-2-PROPEN-1-OL-2-C^{14} (C_1^{14}-Coniferin)



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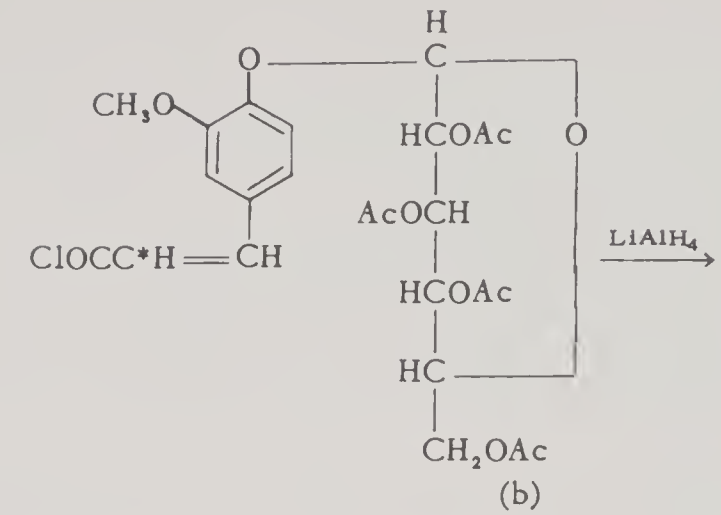

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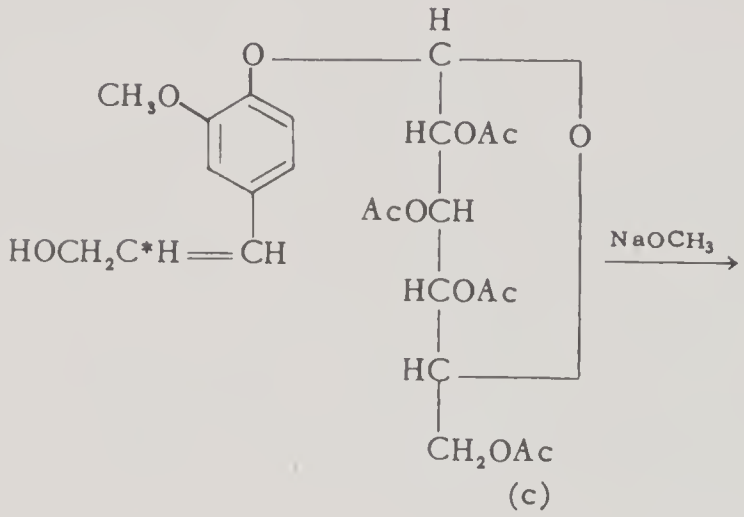

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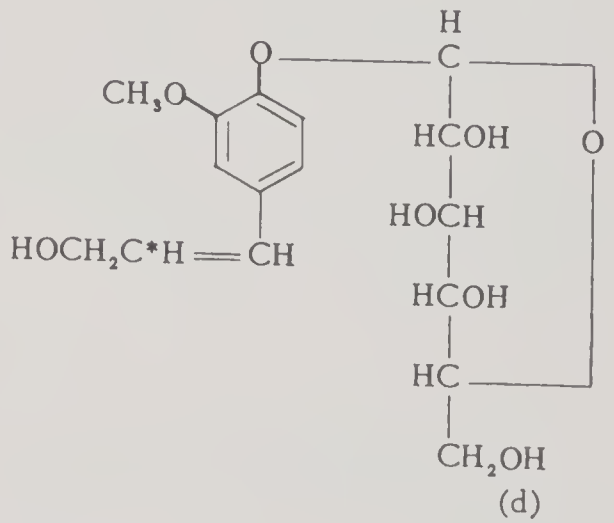


(d)

K. Kratzl and G. Billek, Monatsh. Chem., 84, 406 (1953).

A. Procedure (Note 1)

(a) *O-(Tetra-O-acetyl-β-D-glucosyl)ferulic-α-C*^{14} *Acid,* [*4-(Tetra-O-acetyl-β-D-glucosyloxy)-3-methoxycinnamic-α-C*^{14} *Acid*] (Note 2). A mix-

ture of 0.482 g. of malonic-2-C^{14} acid, 2.3 ml. of pyridine, 2.3 g. of *O*-(tetra-*O*-acetyl-β-D-glucosyl) vanillin and 4 drops of piperidine is heated under reflux for 1.5 hours on a steam-bath. The cooled solution is diluted with 25 ml. of water, and the crystalline product is collected; yield 1.961 g. (82.5%), m.p. 185–200° (Note 3).

(b) *O-(Tetra-O-acetyl-β-D-glucosyl)feruloyl-α-*C^{14} *Chloride.* A mixture of 1.961 g. of the C^{14}-acid, 9 ml. of benzene and 4 ml. of purified thionyl chloride is heated at 75° for 2 hours, then evaporated to dryness. The residue is recrystallized from benzene; yield 1.295 g., m.p. 162–174°. A second crop, obtained by concentrating the mother liquor, raises the yield to 1.438 g. (71%).

(c) *3-[4-(Tetra-O-acetyl-β-D-glucosyloxy)-3-methoxyphenyl]-2-propen-1-ol-2-*C^{14} (Note 4). To a stirred solution of 1.294 g. of the acid chloride in 20 ml. of dry dioxane and 30 ml. of absolute ether, at −15° under pure dry nitrogen, is added 0.250 g. of lithium aluminum hydride in 35 ml. of ether during 1–1.5 hours. The cooled mixture is stirred for 2 hours, then stored overnight at room temperature. The crystalline addition product is collected, washed with ether and mixed with 0.3 g. of boric acid in 15 ml. of water and 30 ml. of benzene, followed by 0.8 ml. of sulfuric acid in 8 ml. of water. The aqueous phase is separated and extracted with benzene (70 ml.), and with chloroform (50 ml.). The extracts are dried and evaporated; yield 0.739 g. (benzene) and 0.093 g. (chloroform) of yellow oil.

(d) *3-(4-β-D-Glucosyloxy-3-methoxyphenyl)-2-propen-1-ol-2-*C^{14} (Note 5). To a stirred solution of 0.822 g. of the crude C_1^{14}-coniferin tetraacetate in 5 ml. of dry chloroform at −15° is added a solution of 0.060 g. of sodium in 10 ml. of methanol. After 20 minutes, the mixture is diluted with 10 ml. of ice water and neutralized with dilute acetic acid, then concentrated to 2 ml. under reduced pressure and cooled to crystallize the product; yield 0.288 g., m.p. 180–185° (Note 6). A second crop of 0.039 g. is obtained by concentrating the mother liquor, making the total yield 35% based on the acid chloride.

B. Notes

1. The conditions for optimum yield were developed by carrying out 15 trial preparations at the 5.18-mmole level without purifying the intermediates.

The preparation by nearly the same procedure at the fractional-mmole scale has been described in detail by Freudenberg[1] (yield 45–50% based on the *O*-(tetra-*O*-acetyl-β-D-glucosyl)ferulic-α-C^{14} acid).

2. The procedure is essentially that of Hann[2] for the Doebner[3] modification of the Knoevenagel reaction.

3. After recrystallization from alcohol, the pure compound melts[2] at 207° (cor.).

4. The method is a modification of that of Allen and Byers (see C_1^{14}-coniferyl alcohol), the acid chloride being more readily reduced than the ester. Freudenberg[1] has employed tetrahydrofuran as solvent and decomposed the reduction complex with dilute acetic acid followed by acetic anhydride in pyridine. An improved reduction procedure employing sodium methoxyborohydride has been described by Fuchs; see 3-(4-β-D-glucosyloxy-3-methoxyphenyl)-2-propen-1-ol-3-C^{14}, Other Preparations.

5. The method of hydrolysis is that of Zemplén (see Note 6). Freudenberg[1] has employed barium methoxide.

6. For the properties of coniferin, see 3-(4-β-D-glucosyloxy-3-methoxyphenyl)-2-propen-1-ol-1-C^{14}.

C. Other Preparations

In a similar manner from malonic-2-C^{14} acid and *O*-(tetra-*O*-acetyl-β-D-glucosyl)syringaldehyde[4] are prepared:[5] 4-(tetra-*O*-acetyl-β-D-glucosyloxy)-3,5-dimethoxycinnamic-α-C^{14} acid (73%, m.p. 160–164°; 165–166° from alcohol), 4-(tetra-*O*-acetyl-β-D-glucosyloxy)-3,5-dimethoxycinnamoyl-α-C^{14} chloride and 3-(4-β-D-glucosyloxy-3,5-dimethoxyphenyl)-2-propen-1-ol-2-C^{14}, (C_1^{14}-syringin), (35%, m.p. 190–191°).

From 4-(tetra-*O*-acetyl-β-D-glucosyloxy)benzaldehyde[6] and malonic-2-C^{14} acid are prepared:[5] 4-(tetra-*O*-acetyl-β-D-glucosyloxy)cinnamic-α-C^{14} acid (79%, m.p. 166–167° from alcohol), 4-(tetra-*O*-acetyl-β-D-glucosyloxy)cinnamoyl-α-C^{14} chloride (84%, m.p. 150–152° from benzene), and 3-(4-β-D-glucosyloxyphenyl)-2-propen-1-ol-2-C^{14} (8%, m.p. 180–182°).

[1]K. Freudenberg and W. Fuchs, Ber., *87*, 1824 (1954).

[2]R. M. Hann, J. Am. Chem. Soc., *56*, 1631 (1934).

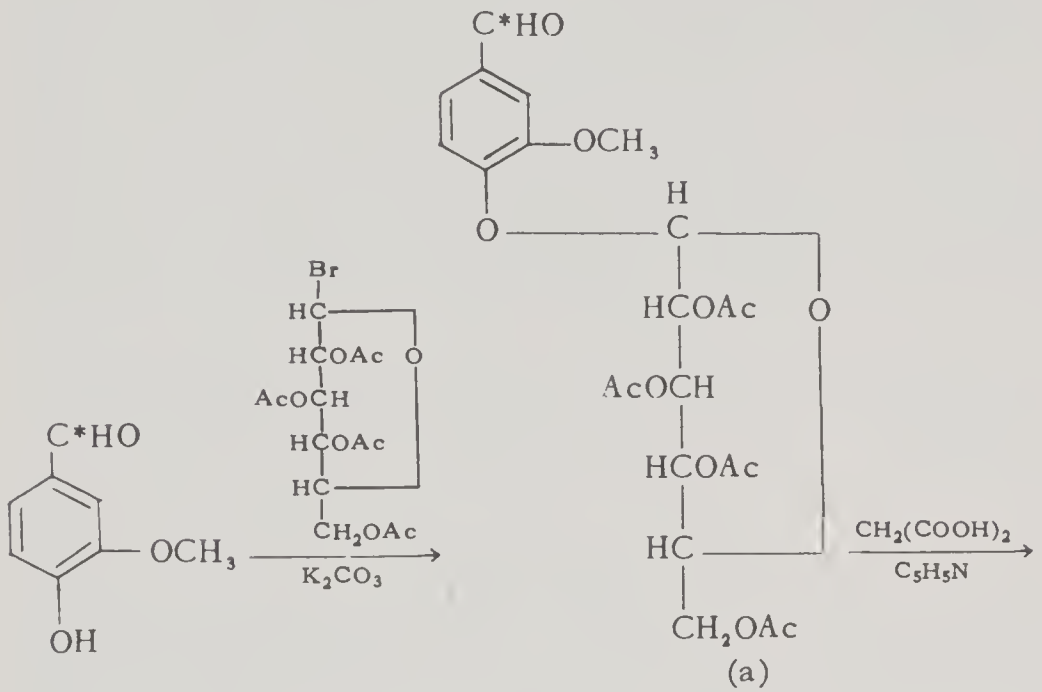
[3]*Organic Reactions*, Vol. I, Wiley, New York, 1942, p. 234; E. Knoevenagel and F. Albert, Ber., *37*, 4481 (1904).

[4]R. L. Shriner and P. McCutchan, J. Am. Chem. Soc., *51*, 2194 (1929); W. Bradley, R. Robinson and G. Schwarzenbach, J. Chem. Soc., *1930*, 793; F. Manthner, J. prakt. Chem., *124*, 318 (1930).

[5]K. Kratzl and G. Billek, Holzforschung, *7*, 66 (1953).

[6]V. Prey, Ber., *74*, 1219 (1941); L. Reichel and R. Schickle, Ann., *553*, 98 (1942).

3-(4-β-D-GLUCOSYLOXY-3-METHOXYPHENYL)-2-PROPEN-1-OL-3-C^{14} (C_1^{14}-Coniferin)



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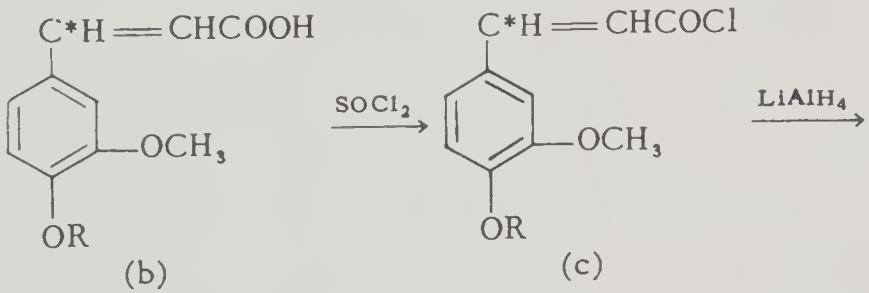

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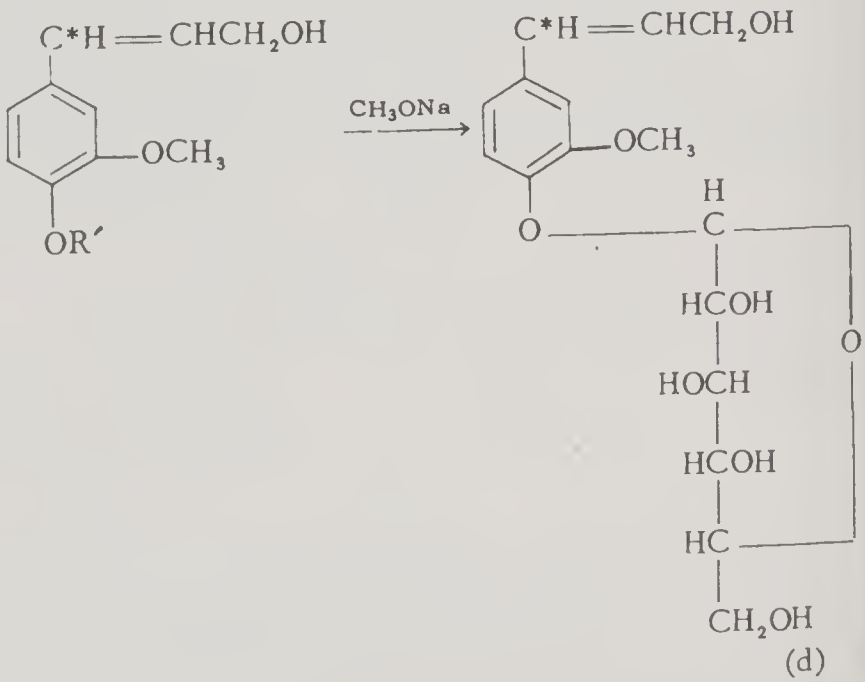


(d)

K. Kratzl and G. Billek, Holzforschung, 7, 66 (1953).

A. Procedure

(a) *4-(Tetra-O-acetyl-β-D-glucosyloxy)-m-anisaldehyde-C^{14}*, [*Tetra-acetyl(C_1^{14}-vanilloside)*]. A solution of 0.218 g. of 4-hydroxy-3-methoxybenzaldehyde-C^{14}, (C_1^{14}-vanillin), 0.585 g. of acetobromoglucose,[1] 0.230 g. of potassium carbonate, 5 ml. of acetone and 3 ml. of water is stored at room temperature for 24 hours. The mixture is evaporated under reduced pressure, and the oil is extracted into benzene solution, which is washed with dilute potassium hydroxide, dried and evaporated; yield 0.397 g. (57%), m.p. 139–143° (Note 2).

(b) *4-(Tetra-O-acetyl-β-D-glucosyloxy)-3-methoxycinnamic-β-C^{14} Acid* (Note 3). A mixture of 0.397 g. of the aldehyde, 0.240 g. of malonic acid, 1 drop of piperidine and 0.5 ml. of pyridine is heated at 100° for 1.5 hours. The mixture is diluted with 5 ml. of water and cooled to crystallize the product; yield 0.399 g. (90%), m.p. 185–200°.

(c) *4-(Tetra-O-acetyl-β-D-glucosyloxy)-3-methoxycinnamoyl-β-C^{14} Chloride.* A mixture of 0.399 g. of the acid, 2 ml. of benzene and 1 ml. of purified thionyl chloride is heated at 75° for 2 hours and evaporated to dryness. The residue is recrystallized from benzene; yield 0.319 g. (77%), m.p. 165–174°.

(d) *3-(4-β-D-Glucosyloxy-3-methoxyphenyl)-2-propen-1-ol-3-C^{14}* (Note 3). To a stirred solution of 0.319 g. of the acid chloride, 5 ml. of anhydrous dioxane and 8 ml. of absolute ether, at −15° in a stream of pure dry nitrogen, is added dropwise a solution of 0.065 g. of lithium aluminum hydride in 12 ml. of ether during 30 minutes, the temperature being maintained below −10°. The mixture is stirred for 2 hours, as the temperature slowly rises to that of the room, and then is stored overnight. The crystalline precipitate is collected, washed with ether and treated with 0.075 g. of boric acid in 4 ml. of water to which is added 5 ml. of benzene. The suspension is shaken with 0.2 ml. of concentrated sulfuric acid in 2 ml. of water, and the aqueous solution is extracted with 35 ml. of benzene and with 20 ml. of chloroform. The combined extract is evaporated under reduced pressure, and the crude partially acetylated coniferin (0.220 g.) is dissolved in 1.5 ml. of chloroform. The solution is cooled to −15°, treated with 0.015 g. of sodium in 2.5 ml. of dry methanol and stored for 20 minutes. The mixture is diluted with 2.5 ml. of ice-water, neutralized with dilute acetic acid, concentrated under reduced pressure to 1 ml. and cooled to crystallize the product; yield 0.070 g. (32%), m.p. 180–185°.

B. Notes

1. The procedure is similar to that used for the preparation of 3-(4-β-D-glucosyloxy-3-methoxyphenyl)-2-propen-1-ol-2-C^{14}.

2. The purity is sufficient for the following steps.

3. An improved preparative method has been recently described by Fuchs;[2] see Other Preparations.

C. Other Preparations

Ferulic-β-C^{14} acid is prepared[3] in a similar manner by condensing 4-hydroxy-3-methoxybenzaldehyde-C^{14} with malonic acid in pyridine containing a little aniline;[4] yield 90% based on C_1^{14}-vanillin.

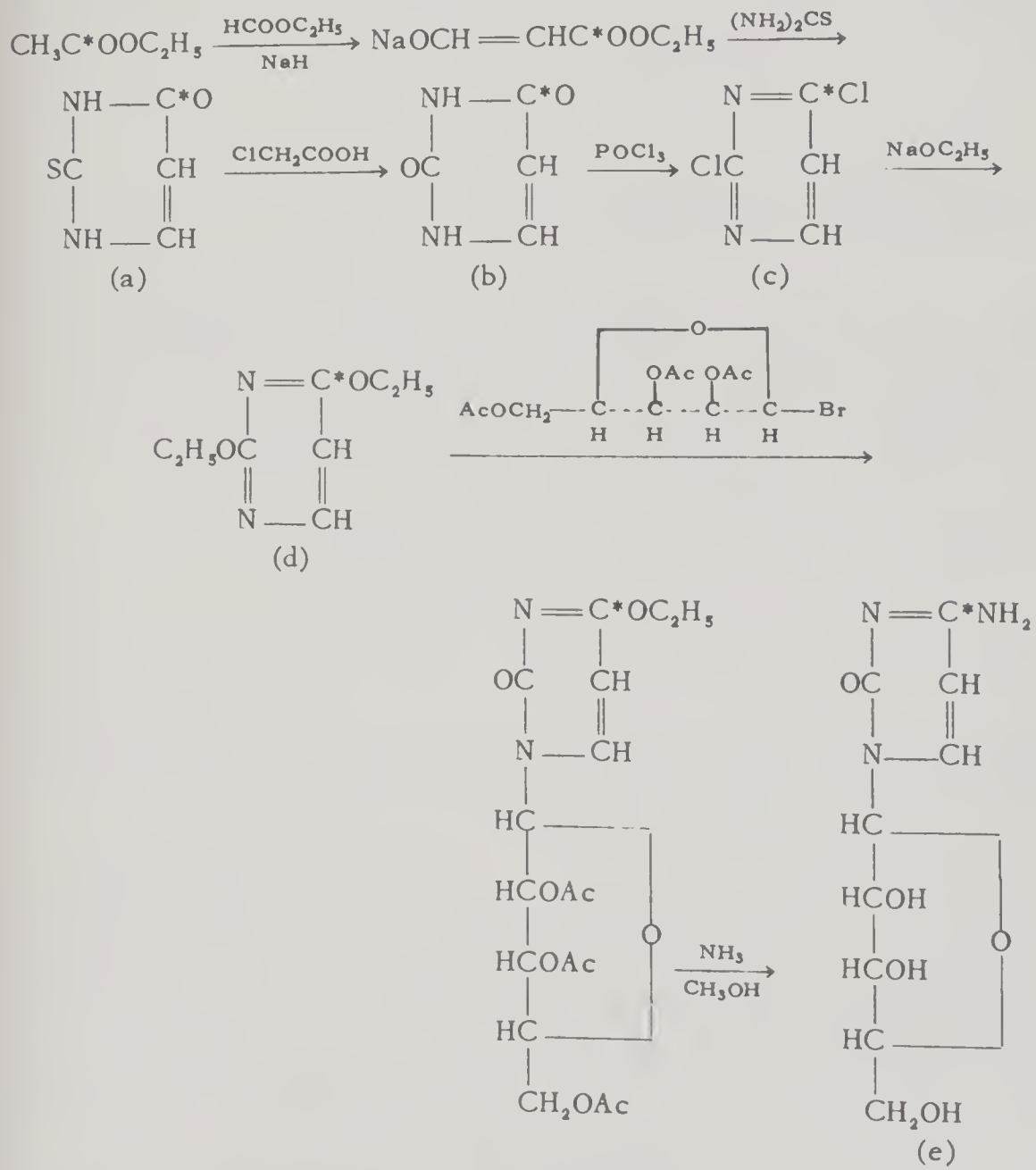
A similar preparation of 4-(tetra-*O*-acetyl-β-L[and D]-glucosyloxy)-*m*-anisaldehyde-C^{14} through use of L[and D]-acetobromoglucose (see 3-(4-β-D-glucosyloxy-3-methoxyphenyl)-2-propen-1-ol-1-C^{14}, Ref. 12) has been described in detail by Freudenberg.[5] Hydrolysis in sodium methoxide (68–92% yield) gave 4-β-L[and D]-glucosyloxy-*m*-anisaldehyde-C^{14}, (C_1^{14}-L [or D]-glucovanillin).

An improved preparation of 3-(4-β-D-glucosyloxy-3-methoxyphenyl)-2-propen-1-ol-3-C^{14} has been described by Fuchs.[2] To a stirred mixture of 1.2 mmoles of sodium methoxyborohydride[6] under nitrogen at -5° is added the 4-(tetra-*O*-acetyl-β-D-glucosyloxy)-3-methoxycinnamoyl-β-C^{14} chloride prepared from 0.278 mmoles of the acid. After 5 minutes the cooling bath is removed, and the mixture is stirred for 30 minutes at 30°; the course of the reduction may be followed by the color reaction with concentrated sulfuric acid and the fading of the yellow coloration with 2,4-dinitrophenylhydrazine. The reaction mixture is diluted with 1 ml. of ice-water, most of the tetrahydrofuran is removed under vacuum, and the residue is taken up in benzene, dried over magnesium sulfate and concentrated in a stream of carbon dioxide. The crystalline residue (0.134 g.) is dissolved in a little chloroform and saponified at -20° with 0.35 ml. of 5% sodium methoxide. After 30 minutes the solution is shaken with somewhat more than the calculated amount of an aqueous suspension of Amberlite XE 64 in the acid form (fine grained IRC 50) containing sufficient methanol to dissolve the chloroform phase; then the neutral reaction mixture is filtered, and the resin is washed with a little warm dilute methanol. The combined filtrate is evaporated under a stream of carbon dioxide, and the product is crystallized from water (radiochemical purity greater than 99.8%). The mother liquor is chromatographed on a cellulose column (Schleicher and Schüll 123a), in methyl ethyl ketone-ethanol-water (75:15:15), making the total yield 63% without the use of carrier (yields in trial reductions were: 85% alcohol, 10% aldehyde and 3% acid). 4-β-D-Glucosyloxy-3-methoxycinnamic-β-C^{14} acid has been prepared in 98% yield by saponification of the starting acid tetraacetate by this procedure. Also, by this method have been prepared:[5] 3-(4-β-D-glucosyloxy-3,5-dimethoxyphenyl)-2-propen-1-ol-3-C^{14}, (C_1^{14}-syringin, 60% yield), and 3-(4-β-D-glucosyloxyphenyl)-2-propen-1-ol-3-C^{14} (excellent yield). The

corresponding sensitive aldehydes may be prepared in good yield by reduction at −80°.

[1]*Organic Syntheses*, Coll. Vol. III, Wiley, New York, 1955, p. 11.
[2]W. Fuchs, Ber., *88*, 1825 (1955).
[3]S. A. Brown and A. C. Neish, Can. J. Biochem. Physiol., *33*, 948 (1955).
[4]F. Vorsatz, J. prakt. Chem., *145*, 265 (1936).
[5]K. Freudenberg and M. Reichert, Ber., *87*, 1834 (1954).
[6]H. C. Brown and E. J. Mead, J. Am. Chem. Soc., *75*, 6263 (1953).

CYTIDINE-4-C^{14}



L. Grossman and D. W. Visser, J. Biol. Chem., *209*, 447 (1954).

A. Procedure

(a) *2-Thiouracil-4-C*^{14} (Note 1). A mixture of 20 g. of ethyl acetate-1-C^{14}, 30 g. of dry ethyl formate and 5.16 g. of sodium hydride is stored overnight at room temperature. The solvent is removed under reduced pressure, and a stirred mixture of the ethyl sodiummalonaldehydate-1-C^{14}, (ethyl sodiumformylacetate-1-C^{14}), 20 g. of dry thiourea and 35 ml. of xylene is refluxed for 6 hours under anhydrous conditions. The solvent is evaporated under reduced pressure, and the residue is dissolved in water and decolorized with carbon; the product is crystallized by acidification with acetic acid. The yield of product recrystallized from water is 19.0 g. (65%), m.p. 340°. The ultraviolet absorption spectrum is the same as that of an authentic sample.

(b) *Uracil-4-C*^{14}. 2-Thiouracil-4-C^{14} is desulphurized with chloroacetic acid as described for thymine-2-C^{14}; also see uracil-2-C^{14}.

(c) *2,4-Dichloropyrimidine-4-C*^{14}. Uracil-4-C^{14} is converted by the procedure of Hilbert,[1] which follows.

A mixture of 100 g. of uracil and 400 ml. of phosphorus oxychloride is heated at 110–120° for 5 hours, then concentrated under reduced pressure, and the residue is slowly treated with 750 g. of crushed ice, with the temperature maintained below 20° (Note 2). The mixture is extracted with ether; the combined extract is washed with sodium carbonate solution, dried over calcium chloride, and concentrated; the product is distilled; yield 90 g. (68%), b.p. 203°, m.p. 61°.

(d) *2,4-Diethoxypyrimidine-4-C*^{14}. 2,4-Dichloropyrimidine-4-C^{14} is converted by the procedure of Hilbert,[2] which follows.

A solution of 40 g. of 2,4-dichloropyrimidine in 200 ml. of absolute ethanol (Note 3) is added slowly to a solution of 12.5 g. of sodium in 200 ml. of absolute ethanol (Note 4). The solution is filtered and concentrated under reduced pressure. The residue is mixed with 100 ml. of 30% sodium hydroxide solution and extracted with ether. The extract is dried over sodium sulfate and concentrated, and the product is distilled; yield 85%, b.p. 224–225°, m.p. 19–20°.

(e) *Cytidine-4-C*^{14}, (*4-Amino-1-β-D-ribosyl-2(1H)-pyrimidinone-4-C*^{14}) (Note 5). The isotopic synthesis is made from 2,4-diethoxypyrimidine-4-C^{14} by a suitable adaptation of the procedure of Howard,[3] which follows. Purification (Note 6) is accomplished by placing 0.150 g. of crude product at pH 3.0 on a 60 × 25-cm. column of Dowex-50 in the acid form, by washing with 100 ml. of water and 250 ml. of 0.2 *N* hydrochloric acid. The product is eluted with 50 ml. of 2.0 *N* hydrochloric acid, and shown to be pure by ionophoresis and ultraviolet absorption (Note 7).

A mixture of 5 g. of crude acetobromoribofuranose (Note 8) and 15 g. of 2,4-diethoxypyrimidine is heated at 65° under anhydrous conditions for 24 hours. The solution is filtered and evaporated at 100° and 10 μ. The residual 9 g. of 4-ethoxy-1-(2,3,5-triacetyl-β-D-ribosyl)-2(1*H*)-pyrimi-

dinone-4-C^{14} is dissolved in 40 ml. of methanolic ammonia (saturated at 0°), and the solution is maintained at 80° for 4 days, then evaporated under reduced pressure. To a solution of the residue in 10 ml. of hot alcohol is added a solution of 5 g. of picric acid in 50 ml. of hot alcohol, and the mixture is cooled. The picrate is collected, recrystallized from water (m.p. 150°) and decomposed with dilute sulfuric acid. The picric acid is removed by ether extraction, the sulfuric acid by precipitation with barium hydroxide. The aqueous solution is concentrated to 5 ml., then diluted with 25 ml. of alcohol and 0.7 ml. of concentrated sulfuric acid, and seeded. The cytidine sulfate is collected and recrystallized from alcohol containing sulfuric acid; yield 0.350 g., m.p. 224° (dec.), $[\alpha]_D^{16} + 35°$ (*c*, 0.366 in 1% aqueous sulfuric acid).

B. Notes

1. The procedure is a modification of the method of Heidelberger and Hurlbert (see ethyl 4,4-diethoxysodiumacetoacetate-1-C^{14}), and of Wheeler.[4]

2. 2,4-Dichloropyrimidine is readily hydrolyzed to uracil. Ether may be added to facilitate decomposition of a phosphorus complex.

3. The reactive 2,4-dichloropyrimidine is dissolved in alcohol without application of heat, for replacement of halogen liberates hydrochloric acid, which hydrolyzes the dialkoxypyrimidine to uracil.

4. If a large excess of sodium ethoxide is used, and there is a delay in working up the reaction mixture, the product contains a relatively large amount of 4-ethoxysodium-2(1*H*)-pyrimidinone,[5] m.p. 305° (cor.) (dec.).

5. The general method is that of Hilbert[6] for synthesizing pyrimidine glycosides.

6. Filter paper ionophoresis at pH 3.7, by the general procedure described by Werkheiser[7] for separating purine and pyrimidine bases, shows the presence of cytosine as well as the desired product.

7. Determination of the molar ultraviolet extinction coefficient[8] shows the purity to be at least 95%.

8. Directions are given by Howard[3] for preparing the starting material. Hydrogenolysis of 1,2,3-triacetyl-5-trityl-D-ribofuranose over palladium, followed by acetylation of the 1,2,3-triacetyl-D-ribose with acetic anhydride in pyridine, gives 1,2,3,5-tetraacetyl-D-ribofuranose, which is halogenated with anhydrous liquid hydrogen bromide. The labile crude product is used without purification.

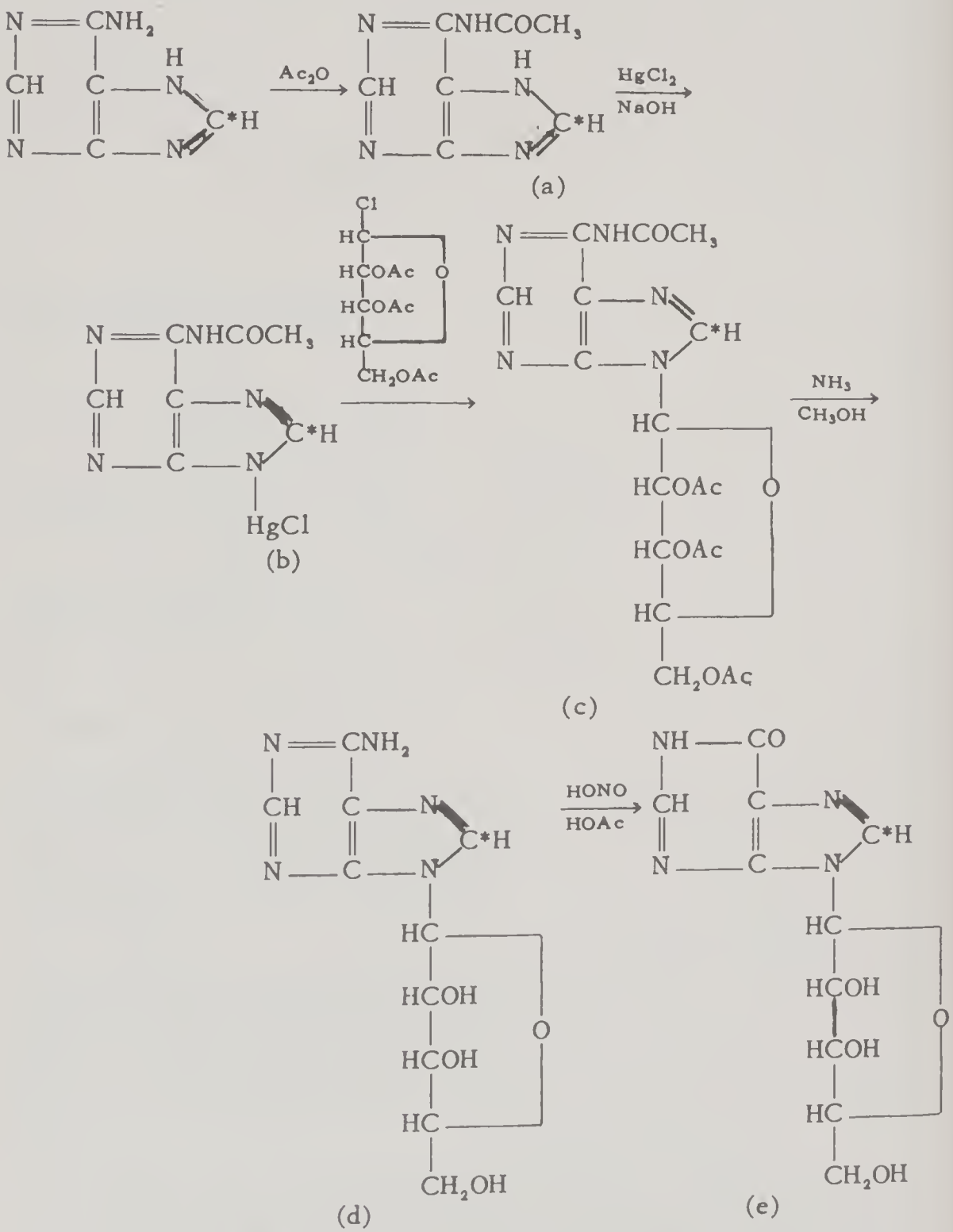
[1]G. E. Hilbert and T. B. Johnson, J. Am. Chem. Soc., *52*, 1152 (1930).
[2]G. E. Hilbert and T. B. Johnson, J. Am. Chem. Soc., *52*, 2001 (1930).
[3]G. A. Howard, B. Lythgoe and A. R. Todd, J. Chem. Soc., *1947*, 1052.
[4]H. L. Wheeler and L. M. Liddle, Am. Chem. J., *40*, 547 (1908).
[5]G. E. Hilbert and E. F. Jansen, J. Am. Chem. Soc., *57*, 552 (1935).

[6]G. E. Hilbert and E. F. Jansen, J. Am. Chem. Soc., *58*, 60 (1936); G. E. Hilbert and T. B. Johnson, J. Am. Chem. Soc., *52*, 4489 (1930).

[7]W. C. Werkheiser and R. J. Winzler, J. Biol. Chem., *204*, 971 (1953).

[8]J. M. Ploeser and H. S. Long, J. Biol. Chem., *178*, 431 (1949).

9-β-D-RIBOFURANOSYLHYPOXANTHINE-8-C^{14} (Inosine-8-C^{14})



B. A. Lowy, J. Davoll and G. B. Brown, J. Biol. Chem., 197, 591 (1952); J. Davoll and B. A. Lowy, J. Am. Chem. Soc., 73, 1650 (1951).

A. Procedure

(a) *6-Acetamidopurine-8-C^{14}*, (*N^6-Acetyladenine-8-C^{14}*). A mixture of 1.35 g. of adenine-8-C^{14} and 8 ml. of acetic anhydride is refluxed for 3 hours and cooled, and the product is collected and washed with ether; yield 1.58 g. (89%); the compound sublimes above 260°.

(b) *6-Acetamido-9-chloromercuri-β-purine-8-C^{14}*. A boiling solution of the purine in 50% ethanol is treated with one equivalent of sodium hydroxide, followed immediately by an alcoholic solution of one molecular proportion of mercuric chloride. The mixture is cooled, and the product is collected by centrifuging and dried under vacuum; yield 88%.

(c) *6-Acetamido-9-(triacetyl-β-D-ribofuranosyl)-β-purine-8-C^{14}*. A stirred suspension of 1.78 g. of the chloromercuri-compound in xylene is dried by slowly distilling until the distillate is clear, then treated with a xylene solution of triacetyl-D-ribofuranosyl chloride, prepared from 1.7 g. of the tetraäcetate (Note 1). The mixture is refluxed for 1 hour, concentrated to about 30 ml. by evaporation (10–20 mm.) and diluted with 150 ml. of petroleum ether (b.p. 30–60°). The precipitate is collected, dried, and extracted with three 20-ml. portions of warm chloroform. The extract is washed with 30% aqueous potassium iodide solution and water, then dried over sodium sulfate and evaporated to dryness at 10–20 mm.

(d) *6-Amino-9-β-D-ribofuranosyl-β-purine-8-C^{14}*, (*Adenosine-8-C^{14}*). The syrupy tetraacetyl compound is dissolved in 25 ml. of boiling methanol, and the solution is cooled quickly to room temperature. An equal volume of methanol, saturated with ammonia at 0°, is added, and the mixture is stored at 0° for 18 hours. The residue, obtained by evaporation to dryness at 10–20 mm., is crystallized from water; yield 0.307 g. (27%), after working up the mother liquors. A sample recrystallized from water melts at 235–236° (Note 2).

(e) *9-β-D-Ribofuranosylhypoxanthine-8-C^{14}* (Note 3). Adenosine-8-C^{14} is deaminated by the action of sodium nitrite and acetic acid (see 9-β-D-ribofuranosylguanine-2-C^{14}), and the product is purified through its lead salt. The salt is prepared by treating the dilute acid reaction mixture with an excess of lead acetate solution and aqueous ammonia. The precipitate is collected and washed, then dissolved in 30 ml. of 20% acetic acid, and the solution is saturated with hydrogen sulfide. The filtered solution is evaporated to dryness, and the crude product is recrystallized from 80% alcohol (Note 4).

B. Notes

1. The total volume of xylene is 85 ml. Moisture is excluded from the system, and care is taken to avoid local overheating of separated mate-

rial on the flask walls. The starting material is prepared, by the method of Davoll,[1] from tetraacetyl-D-ribofuranose, which is obtained according to Bredereck[2] or Zinner.[3]

2. $[\alpha]_D^{28}$-65.5° (*c*, 0.6% in water). The product may be isolated as the picrate (m.p. 181–185° dec.) by treating the aqueous solution of the residue with ethanolic picric acid. A solution of 0.40 g. of the picrate in 80 ml. of hot water is treated with two portions of Dowex-1 anion-exchange resin in the chloride form. The filtrate is treated with one equivalent of sodium hydroxide, and concentrated to 4 ml. to give 0.176 g. (82% yield) of adenosine, m.p. 236°. The identity of the synthetic and natural nucleosides has been established by paper chromatography;[4] R_f 0.42 with 1-butanol-diethylene glycol-water in an ammonia atmosphere.

3. The procedure is a modification of the method of Levene,[5] which gave nearly quantitative yields.

4. The product crystallizes from water as a dihydrate, m.p. 90°, but from 80% alcohol as anhydrous needles, m.p. 218° (dec.) (rapid heating).

C. Other Preparations

The following analogues are prepared from 2,6-diaminopurine-2-C^{14} by the same procedures; each exhibits a single spot on a paper chromatogram:[1]

2,6-Diacetamidopurine-2-C^{14}, 83% yield, m.p. 295–300° (dec.); recrystallization from ethanol-acetone-water raises the melting point to 295–306° (dec.).

2,6-Diacetamido-9-chloromercuri-β-purine-2-C^{14}, 83% yield.

2,6-Diacetamido-9-(triacetyl-β-D-ribofuranosyl)-β-purine-2-C^{14}, 57% crude yield, a yellow glass.

2,6-Diamino-9-β-D-ribofuranosyl-β-purine-2-C^{14}, 52% yield, m.p. 245–247°. A solution of 2.56 g. of the pentaacetate and 20 ml. of warm methanol is mixed with a solution of 0.40 g. of sodium in 40 ml. of methanol, and left at room temperature for 4 hours. The mixture is refluxed for 30 minutes, neutralized with acetic acid and evaporated to dryness at 10–20 mm. The residue is decolorized with charcoal and crystallized from water to give 0.76 g. of product. Recrystallization from water raises the melting point to 248°; $[\alpha]_D^{28}$-40.5° (*c*,0.7% in 0.1 *N* hydrochloric acid). The ultraviolet absorption spectrum is described.

9-β-D-Ribofuranosylisoguanine-2-C^{14}, (C_1^{14}-crotonoside, C_1^{14}-isoguanosine, or 2-hydroxyadenosine-2-C^{14}); yield 57%. To a solution[6] of 0.30 g. of 2,6-diamino-9-β-D-ribofuranosyl-β-purine-2-C^{14} and 0.45 g. of sodium nitrite in 5 ml. of water cooled to 50° is added 0.45 ml. of glacial acetic acid. The mixture is maintained at 50° for 5 minutes, then dissolved in 15 ml. of warm water, and the product is isolated through the lead salt.

The melting point lies between 237° and 252° (dec.) depending upon the rate of heating. Paper chromatography[1] in 1-butanol-diethylene glycol-water mixture with an ammonia atmosphere exhibits only one spot. The picrate decomposes at 212–225°.

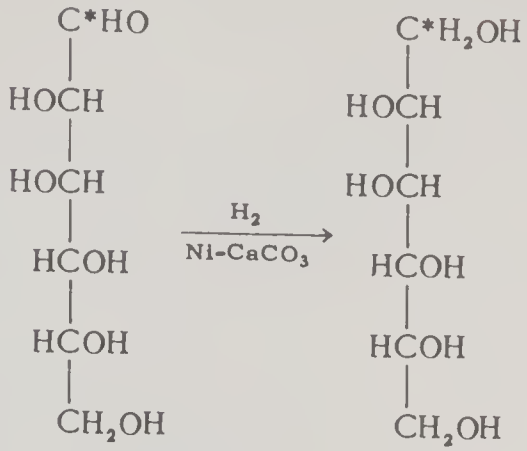
2-Acetamido-6-amino-9-β-D-ribofuranosyl-β-purine-2-C^{14}. A hot solution of 1.96 g. of crude 2,6-diacetamido-9-(triacetyl-β-D-ribofuranosyl)-β-purine-2-C^{14} in 20 ml. of methanol is cooled to room temperature, diluted with 40 ml. of methanol which has been saturated with ammonia at 0°, then stored overnight at 0° and finally evaporated to dryness. The residue is decolorized with charcoal and crystallized from 15 ml. of water; yield 1.0 g. (77%). The melting point is indefinite between 140° and 150°, and is not altered by recrystallization from water.

9-β-D-Ribofuranosylguanine-2-C^{14}, (Guanosine-2-C^{14}). A solution of 0.32 g. of 2-acetamido-6-amino-9-β-D-ribofuranosyl-β-purine-2-C^{14} and 0.80 g. of sodium nitrite in 2.5 ml. of hot water is cooled quickly to room temperature and shaken with 0.80 ml. of glacial acetic acid. After about 1 hour, the solution is diluted with an equal volume of water and stored overnight at room temperature. The solution then is diluted with 20 ml. of water and treated with an excess of lead acetate solution and aqueous ammonia. The precipitate is collected, washed and dissolved in 30 ml. of 20% acetic acid, and the solution is saturated with hydrogen sulfide, filtered and evaporated to dryness. The residue is refluxed for 1 hour with a solution of 0.10 g. of sodium in 15 ml. of methanol. The mixture is diluted to a clear solution with water and refluxed for 45 minutes, then neutralized with acetic acid and evaporated to dryness. The residue is dissolved in 14 ml. of water and seeded to crystallize; yield 0.28 g., 71%. Recrystallization from 7 ml. of water gives 0.223 g., the compound chars without melting at 235°; $[\alpha]_D^{26}$ −72° (*c*,1.4% in 0.1 *N* sodium hydroxide). The dihydrate loses water at 110°. One gram dissolves in 1320 ml. of water at 18°, or 33 ml. at the boiling point. The ultraviolet absorption spectrum is described.

The preparation of a number of 9-glycosyl-β-purines is described by Davoll and Lowy.[7]

[1]J. Davoll, B. Lythgoe and A. R. Todd, J. Chem. Soc., *1948*, 967.
[2]H. Bredereck and E. Hoepfner, Ber., *81*, 51 (1948).
[3]H. Zinner, *ibid.*, *83*, 153 (1950).
[4]E. Vischer and E. Chargaff, J. Biol. Chem., *176*, 703 (1948).
[5]P. A. Levene and R. S. Tipson, J. Biol. Chem., *111*, 313 (1935); P. A. Levene and W. A. Jacobs, Ber., *43*, 3150 (1910).
[6]J. Davoll, J. Am. Chem. Soc., 73, 3174 (1951).
[7]J. Davoll and B. A. Lowy, J. Am. Chem. Soc., 74, 1563 (1952).

D-MANNITOL-1-C^{14}



H. S. Isbell and J. V. Karabinos, J. Research Nat. Bur. Standards, *48*, 438 (1952).

A. Procedure

A mixture of the crude, noncrystalline D-mannose-1-C^{14}, prepared from 1 mmole of D-mannono-γ-lactone-1-C^{14}, 2 ml. of alcohol, 3 ml. of water and 0.1 g. of calcium carbonate is hydrogenated over 0.5 g. of Raney nickel for 2 hours at 125° and 1000 p.s.i. The solution is filtered and passed through a deionizing column (Note 1), and the combined effluent and washings are lyophilized. The residue is dissolved in a minimal volume of hot methanol, and the solution is diluted with an equal volume of isopropyl alcohol, seeded and stored for a few days to crystallize; yield 0.117 g., m.p. 165°. A second crop of 0.098 g. is obtained by recrystallizing 0.100 g. of carrier from the mother liquor, making the total radiochemical yield 80% (Note 2).

B. Notes

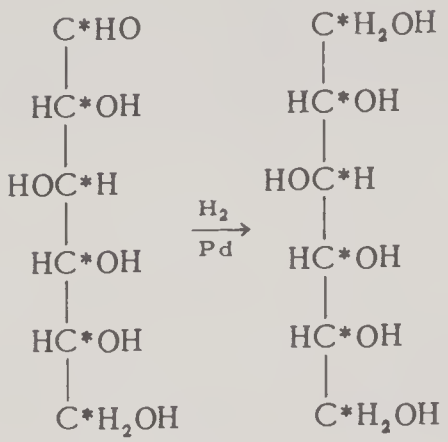
1. A 1.4 × 25-cm. column containing equal parts of Amberlite IR-100H and Duolite A-4 is used.

2. Directions are given by Isbell for converting the product to D-fructose-1,6-$C^{14}_{1/2}$ (54.4% radiochemical yield) by microbiological oxidation. The crystallization of fructose and the degradation and assay of the terminal carbons have been described by Frush.[1] Alkaline degradation in the presence of molecular oxygen[2] is adaptable to a semimicro-scale for the assay of C-1 of any simple aldose or 2-ketose. For assay of the other terminal carbon, the lower sugar acid formed is degraded further to formaldehyde with sodium periodate; see D-arabinose-5-C^{14}.

[1]H. L. Frush and H. S. Isbell, Atomic Energy Commission Report, NBS-2308; Nuc. Sci. Abstracts, 7, 2767 (1953).

[2]H. S. Isbell, J. Research Nat. Bur. Standards, *29*, 227, (1942).

D-SORBITOL-C_6^{14}



M. R. Stetten and D. Stetten, Jr., J. Biol. Chem., *193*, 157 (1951).

A. Procedure

D-Glucose-C_6^{14} (10 g., Note 1) in 30 ml. of 20% aqueous alcohol is hydrogenated over palladium (Note 2) with shaking at 105–108° and 1450 p.s.i. for 5 hours. The filtered solution is evaporated to dryness at reduced pressure, and the residue is extracted with hot pyridine. The extract is concentrated to crystallize two crops of D-sorbitol-C_6^{14} pyridine complex (Note 3); then two more crops are obtained from the mother liquor with the aid of 2 g. of carrier. The combined crops of complex are twice recrystallized from pyridine and dried under vacuum at room temperature over sulfuric acid; yield 9.244 g. The pyridine of crystallization is removed quantitatively by heating over sulfuric acid at 80° for 48 hours at 1 mm. The yield of anhydrous D-sorbitol-C_6^{14} is 6.441 g. (Note 4), and the preparation is free from glucose (Note 5).

B. Notes

1. The starting material may be prepared from uniformly labeled starch by the procedure of Stetten[2] or Wick.[2]

2. The catalyst is prepared by hydrogenating 2 g. of $PdCl_2 \cdot 2H_2O$ for 15 minutes at room temperature at 1500 p.s.i., then washing with five portions of water to remove hydrochloric acid.

3. D-Sorbitol is very soluble in hot pyridine; but only slightly soluble at 0° (1 g./100 ml.), crystallizing with one molecule of pyridine. Reducing sugars and mannitol are quite soluble under these conditions, while other hexitols are much less soluble in pyridine and separate without complex formation with the solvent. The complex liberates pyridine in the presence of moisture.

4. The anhydrous compound[3] melts between 89 and 93° (cor.), distills at 200° (1 μ), and may be recrystallized from ethyl lactate, ethanol or pentylamine by the addition of petroleum ether.

5. The recrystallized glucosazone, prepared from a mixture of 0.200 g. of the D-sorbitol-C_6^{14} and 0.040 g. of D-glucose, was nonradioactive.

C. Other Preparations

The preparation of D-sortibol-C_6^{14} by hydrogenation of D-glucose-C_6^{14} over Raney nickel has been reported by Wick.[4] Paper chromatography and radioautography indicated that the product was free from radioactive contaminants.

The preparation of D-sorbitol-1-C^{14} by reduction of D-glucose-1-C^{14} (0.050 g.) over Raney nickel has been described by Hers;[5] yield less than 20%, because most of the product could not be eluted from the catalyst. Purification was accomplished by chromatography[6] on Whatman No. 1 filter paper with water-saturated phenol as solvent. The preparation has been reported by Brown;[7] purification was accomplished through formation of the pyridine complex, recrystallization to constant specific activity and regeneration of the free sugar.

The preparation of L-sorbose-C_6^{14} and L-sorbose-6-C^{14}, m.p. 162–164°, by enzymatic oxidation[8] of D-sorbitol-C_6^{14} and D-sorbitol-6-C^{14} has been reported by Burns.[9] Paper chromatography,[6] radioautography and ion exchange chromatography[10] (of the borate complex) showed the product to be radioactively homogeneous. Degradation studies[9] indicated that there was no isotopic rearrangement. The preparation of D-sorbitol-C_6^{14} by hydrogenation of D-glucose-C_6^{14} over Raney nickel in aqueous alkaline medium, and the oxidation to L-sorbose-C_6^{14}, m.p. 163–165°, have been reported also by Bothner-By.[8]

[1]M. R. Stetten and D. Stetten, Jr., J. Biol. Chem., *187*, 241 (1950).

[2]A. N. Wick, M. E. Blackwell and N. Hillyard, Arch. Biochem. Biophys., *32*, 274 (1951).

[3]H. H. Strain, J. Am. Chem. Soc., *56*, 1756 (1934).

[4]A. N. Wick, M. C. Almen, and L. Joseph, J. Am. Pharm Assoc., Sci. Ed., *40*, 542 (1951).

[5]H. G. Hers, J. Biol. Chem., *214*, 373 (1955); J. V. Karabinos and A. T. Ballun, J. Am. Chem. Soc., *75*, 4501 (1953).

[6]S. M. Partridge, Biochem. J., *42*, 238 (1948).

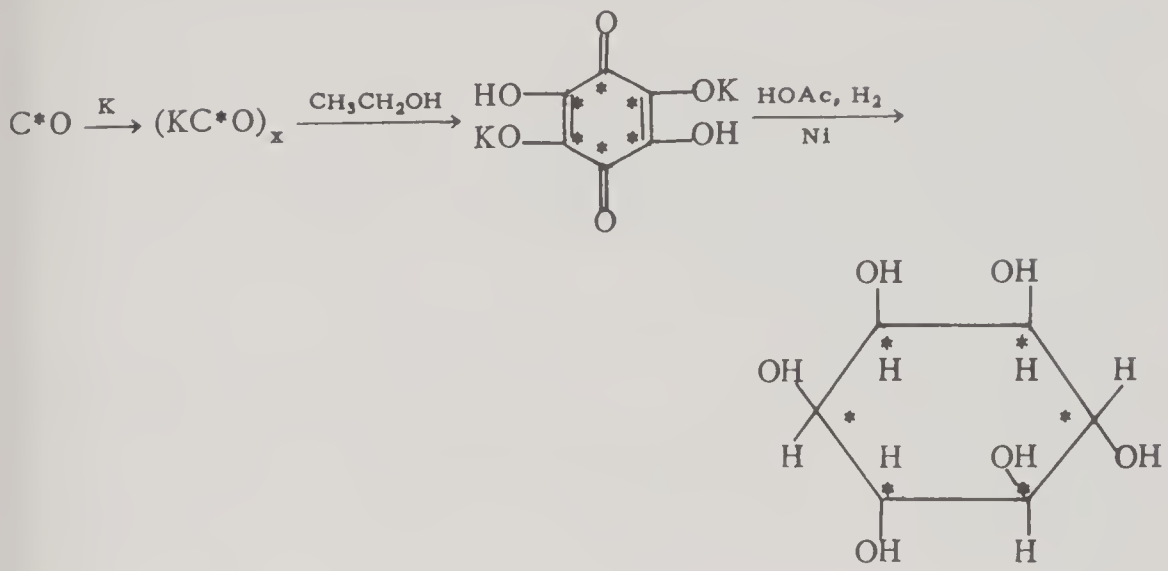
[7]S. A. Brown and A. C. Neish, Can. J. Biochem. Physiol., *32*, 170 (1954).

[8]A. A. Bothner-By, M. Gibbs and R. A. Anderson, Science, *112*, 363 (1950).

[9]J. J. Burns, E. H. Mosbach, S. Schulenberg and J. Reichenthal, J. Biol. Chem., *214*, 507 (1955).

[10]J. X. Khym and L. P. Zill, J. Am. Chem. Soc., *74*, 2090 (1952).

Meso-INOSITOL-C_6^{14}



R. C. Anderson, Atomic Energy Commission Report, AECU-956; Nuc. Sci. Abstracts, *4*, 6590 (1950); R. Anderson and H. Prosser, Nucleonics, 7 (4), 47 (1950).

A. Procedure (Note 1)

Potassium carbonyl-C^{14}, prepared by breaking a sealed bulb containing potassium in an atmosphere of carbon-C^{14} monoxide, is hydrolyzed by condensing absolute alcohol on it at −75° and warming the mixture to room temperature (Note 2). The dipotassium tetrahydroxybenzoquinone-C_6^{14} in 40% ethanol solution (Note 3) is acidified with acetic acid and hydrogenated at 2500 p.s.i. over Raney nickel (Note 4). The solution of cyclitols is filtered and evaporated to dryness under vacuum. Upon treatment of the residue with methanol, the syrup slowly dissolves and crystallization follows. The scyllitol-C_6^{14}, (1,3,5/2,4,6-cyclohexanehexol-C_6^{14}), is collected, recrystallized by treating a filtered aqueous solution with sufficient methanol to make the concentration 50%, and dried at 100° for 1 hour at 1 mm., m.p. 350–354° (Note 5).[1]

The mother liquor is diluted with methanol to an 80% mixture, and the *meso*-inositol-C_6^{14}, (1,3/2,4,5,6-cyclohexanehexol-C_6^{14}), is collected and recrystallized from water-methanol, m.p. 219–220° (Note 6).[1] The yield is 12% based on carbon-C^{14} monoxide.

B. Notes

1. The procedure is adapted from the method of Nietzki[2] for preparing tetrahydroxyquinone. The configuration notation is that of Anderson and Wallis.[1]

2. The reaction mixture is explosive in contact with air.[2]

3. The salts of tetrahydroxyquinone in water solution change over to the corresponding salts of croconic acid with loss of carbon dioxide and water.[3]

4. The method is a modification of that described by Anderson,[1] in which disodium tetrahydroxybenzoquinone was reduced in stannous chloride-hydrochloric acid solution, and the resulting hexahydroxybenzene was hydrogenated over Raney nickel[4] at 125° in ethanol at 1910 p.s.i., forming a mixture of cyclitols. The method of working up the mixture is that described by Anderson[1] for a 15-g. run.

5. The hexaacetate, m.p. 300–301°, is prepared[1] by refluxing the carbinol with acetic anhydride in the presence of sulfuric acid catalyst. Purification is accomplished by vacuum sublimation at 200°, to free it of bound acetic acid, followed by recrystallization from methanol.

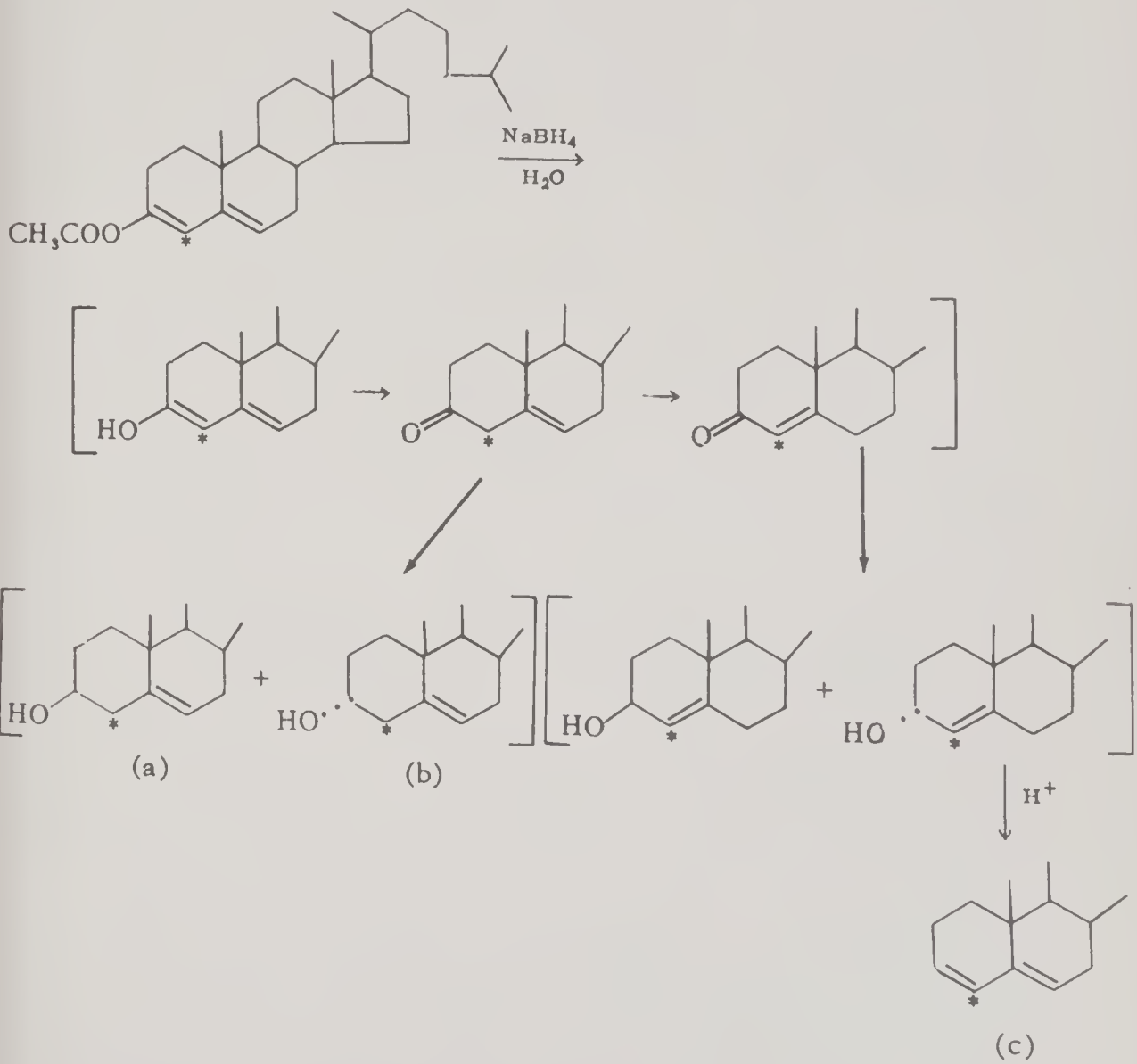
6. Sublimation[1] at 200° (0.1 μ) raises the melting point to 224–225°. The hexaacetate melts at 213–214°.

[1]R. C. Anderson and E. S. Wallis, J. Am. Chem. Soc., *70*, 2931 (1948).
[2]R. Nietzki and T. Benckiser, Ber., *18*, 1833 (1885).
[3]O. Gelormini and N. E. Artz, J. Am. Chem. Soc., *52*, 2483 (1930).
[4]*Organic Syntheses*, Vol. 21, Wiley, New York, 1941, p. 15.

STEROIDS

CHOLESTEROL-4-C^{14}

METHOD I

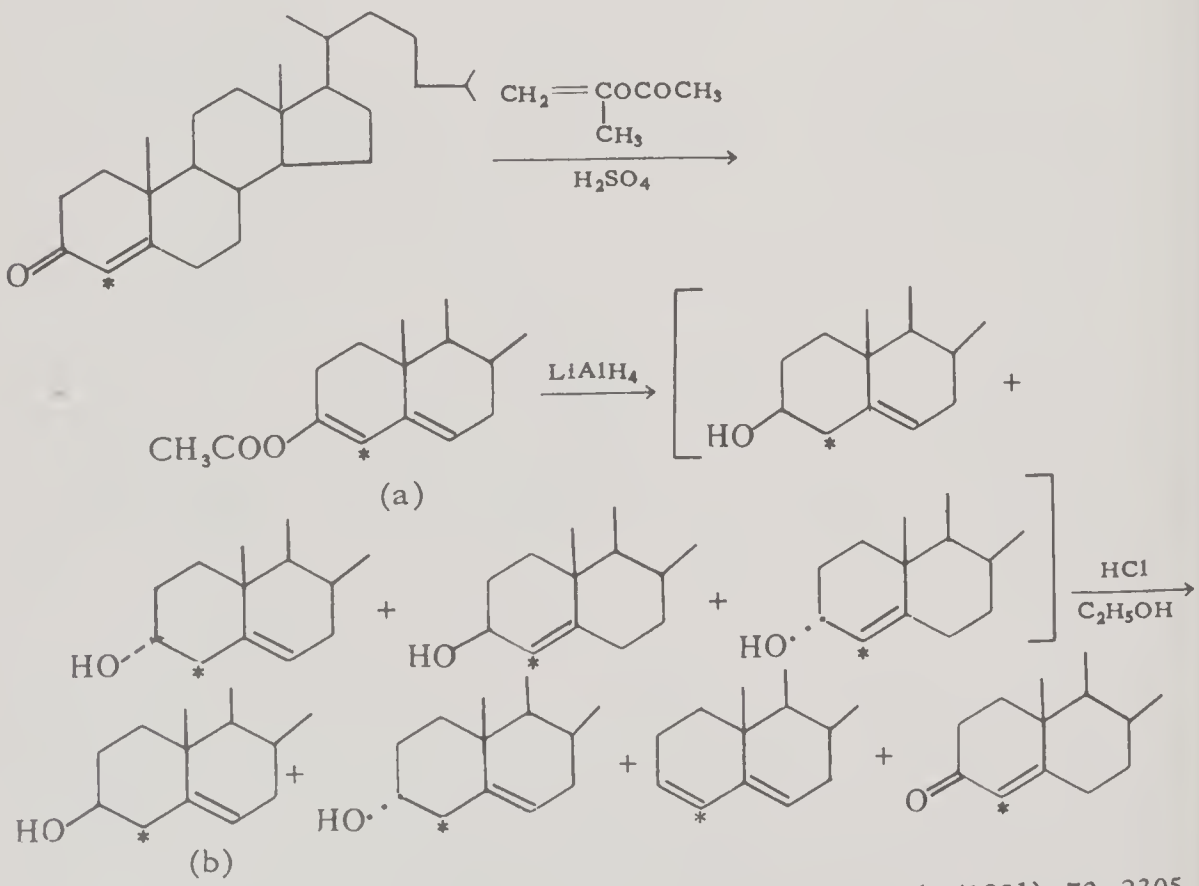


B. Belleau and T. F. Gallagher, J. Am. Chem. Soc., 73, 4458 (1951).

A. Procedure (Note 1)

A solution of 0.500 g. of 3-acetoxy-3,5-cholestadiene-4-C^{14}, (4-cholesten-3-one-4-C^{14} enol acetate, see Method II) in 200 ml. of redistilled 95% ethanol is added at 5° to a cooled, stirred solution of 1 g. of sodium borohydride in 25 ml. of 70% aqueous ethanol and stored at 5° for 2 hours (Note 2). The solution is heated to boiling, 25 ml. of 5% aqueous sodium hydroxide is added, and the ethanol is removed under vacuum. The residue is acidified with dilute hydrochloric acid and extracted with ether. After evaporation of solvent, the residue is refluxed for 2 hours with 30 ml. of ethanol containing 4 drops of hydrochloric acid (Note 3). The solution is evaporated to dryness under vacuum, and an alcoholic solution of the residue is treated with an excess of digitonin in ethanol. The digitonide is collected, washed with ether and dissolved in pyridine, and the digitonin is precipitated with ether and filtered off. The filtrate is washed with dilute hydrochloric acid and water, dried over sodium sulfate and evaporated; yield 0.385 g. (85%), m.p. 138–145°. Purification is effected by chromatographic partition on 40 g. of silica gel (see Method II). Elution with ether-petroleum ether (1:3) gives 12 mg. of solid, m.p. 130–140°, followed by 0.326 g. of product, m.p. 144–148° (Notes 4 and 12).

METHOD II



W. G. Dauben and J. F. Eastham, J. Am. Chem. Soc., 73, 3260 (1951); 72, 2305 (1950)

A. Procedure

(a) *3-Acetoxy-3,5-cholestadiene-4-C*^{14}, *(4-Cholesten-3-one-4-C*^{14} *Enol Acetate*) (Note 5). A mixture of 5.0 g. (13 mmoles) of 4-cholesten-3-one-4-C^{14}, 5 ml. of isopropenyl acetate and 0.02 ml. of concentrated sulfuric acid is refluxed for 1 hour. One ml. of distillate is removed under reduced pressure, and the mixture is again refluxed for 1 hour. Anhydrous sodium acetate (0.1 g.) is added, and the mixture is concentrated under reduced pressure. The residual oil is dissolved in a few ml. of chloroform and decanted from the sodium acetate into 65 ml. of methanol. The oil is just dissolved by adding chloroform to the boiling mixture, which is then set aside to crystallize by slow cooling; yield 4.8 g. (87%), m.p. 76–78°.

(b) *Cholesterol-4-C*^{14}. To a boiling mixture of 2.0 g. (53 mmoles) of lithium aluminum hydride and 50 ml. of anhydrous tetrahydrofuran under nitrogen is added slowly a solution of 1.969 g. (4.6 mmoles) of 4-cholesten-3-one-4-C^{14} enol acetate (Note 6) in tetrahydrofuran (Note 7) over a period of 1 hour. The mixture is heated for 3 hours longer, then decomposed with 70 ml. of 20% sulfuric acid (Note 8) and extracted with 60 ml. of ether. The combined extract is washed with saturated sodium potassium tartrate solution, dried over magnesium sulfate and evaporated to give 1.780 g. of amorphous residue. To a solution of this residue in 50 ml. of hot 90% ethanol is added a hot solution of 2.5 g. of digitonin in 100 ml. of the same solvent, and the mixture is stored at 0° for 12 hours. The collected digitonide (3.03 g.) is dissolved in 35 ml. of anhydrous pyridine, to which 200 ml. of ether is then added. The suspension is centrifuged and washed with two 100-ml. portions of ether. The combined solutions are filtered, washed with 20% sulfuric acid and saturated sodium chloride solutions, then dried and evaporated. The residue (0.73 g.) is dissolved in 50 ml. of ethanol containing 1.5 ml. of concentrated hydrochloric acid, and the solution is refluxed for 6 hours (Note 9). The solution is cooled, diluted with 150 ml. of ether and washed with water, sodium bicarbonate and saturated sodium chloride solutions, then dried over potassium carbonate and evaporated. The residue is dissolved in hexane and chromatographed on a column of aluminum oxide (Note 10). Elution with the solvent sequence: a) hexane, b) ether (15v%) in hexane, c) ether (25v%) in hexane yields the following compounds: a) 0.097 g. of 3,5-cholestadiene-4-C^{14}, m.p. 65–70°; b) 0.036 g. of 4-cholesten-3-one-4-C^{14}, m.p. 86–87°; c) 0.577 g. of cholesterol-4-C^{14}, m.p. 145–147° (Notes 11 and 12).

The mother liquor from the digitonide precipitation is acidified with 5 ml. of concentrated hydrochloric acid, refluxed for 6 hours, diluted with 500 ml. of ether and processed as above. The crude yield is 1.01 g., and chromatographic separation gives: 0.129 g. of 3,5-cholestadiene-4-C^{14}; 0.478 g. of 4-cholesten-3-one-4-C^{14}, m.p. 87.0–87.5°; and 0.298 g. of epicholesterol-4-C^{14}, m.p. 132–137° (Note 13).

B. Notes

1. The preparation was developed for isotopic labeling, but was reported originally in the nonisotopic form. At the same time directions for an isotopic preparation were published by Schwenk.[1]

2. As sodium borohydride does not reduce esters, this reduction of an enol ester must be by a different mechanism than is found with lithium aluminum hydride. Although carbonyl groups are completely reduced by sodium borohydride in anhydrous pyridine, this enol-acetate is recovered unchanged by such treatment, thereby indicating that saponification is the first step. The enol form of the α,β unsaturated ketone must rearrange in two distinct stages, for, in the presence of excess reducing agent, the Δ^4-stenols are formed only to the extent of 1–3%.

3. The isomeric allylic Δ^4-sterols are destroyed by dehydration (see Method II).

4. Recrystallization from alcohol gives 0.317 g. of cholesterol melting at 146–148°. The combined mother liquor from the digitonide and ether washings is diluted with ether and filtered. The filtrate is evaporated, and the resulting oil is separated by chromatographic partition, as above, giving 17 mg. of crude 3,5-cholestadiene-4-C^{14} and 32 mg. (7.4%) of epicholesterol-4-C^{14}, (c) and (b), respectively (Note 12).

Cholesterol has been prepared in 37% yield by treating cholestenone itself with *t*-butylmagnesium chloride, followed by hydrolysis and reduction with aqeuous alcoholic sodium borohydride solution. Grignard and Blanchon[2] have shown that ketones readily enolize and form salts in the presence of *t*-alkylmagnesium halides. The formation of such a salt effects the requisite migration of the double bond from Δ^4 to Δ^5, without the need to form an ester of cholestenone; hydrolysis then forms the ketone without rearrangement of the double bond.

5. Acetylation of the enol is performed with the unique and mild reagent, isopropenyl acetate, by the method of Hagemeyer and Hull.[3] The reagent reacts with all enolizable carbonyl compounds, and the acetylation is driven to completion by distillation of the acetone formed. By heating in the presence of acid catalysts, the reagent decomposes into ketene and acetone. The enol acetate also has been prepared (90% yield) by the method of Westphal,[4] employing acetic anhydride and acetyl chloride.

6. It has been shown that reduction of 4-cholesten-3-one forms[5] nearly equal amounts of the α- and β-4-cholesten-3-ols, while reduction of 5-cholesten-3-one with this same reagent forms[6] the isomeric epicholesterol and cholesterol in the ratio 5% to 90%.

7. Quantity and concentration of hydride, mode of addition, time and temperature variation (−15° to 100°) have little effect on the course of

this thoroughly studied reaction. The inverse reduction prevents the formation of the Δ^4-isomeric stenols, and the cholesterol, epicholesterol and cholestenone may be separated directly by chromatography.

8. The decomposition should be done cautiously and with cooling.

9. The treatment with dilute alcoholic hydrochloric acid dehydrates the allylic Δ^4-sterols, 4-cholesten-3α-ol (4%) and 4-cholesten-3β-ol (4%), converting them into 3,5-cholestadiene. Cholesterol and epicholesterol are little affected under these mild conditions.

10. Merk and Co., reagent grade (25 g.).

11. Recrystallization gives a product melting at 146–148°. The yield is 50% based on recovered 4-cholesten-3-one-4-C^{14}, including the material recovered by oxidation of all other sterol fractions and hydrolysis of residual products from the preparation of the enol acetate. Schwenk[1] has reported a 35% yield by this procedure, but found that the yield could be raised to 71% by reducing with sodium borohydride (see Method I).

12. A sample of cholesterol-4-C^{14} (6.5 μc./mg.) after 18 months of self-irradiation was found[7] to have decomposed to the extent that 40% of the material was no longer precipitable by digitonin. When C^{14}-cholesterol is stored in the presence of air, it is oxidized on and about the double bond, forming chiefly the epimeric 7-hydroxy, the 7-keto and the 5α,6β-dihydroxy derivatives; the reaction requires both radiation and oxygen.[8]

13. Epicholesterol-4-C^{14} may be purified immediately before use by the procedure of Harold.[9] β-Steroids are precipitated with digitonin, and the supernatant solution is evaporated. The residue is dissolved in pyridine, and the free digitonin is precipitated with ether. The supernatant solution is evaporated, and the residue is twice purified by chromatography in hexane solution on alumina and elution with 15% ether in hexane; m. p. 140°.

C. Other Preparations

The preparation of cholesterol-3-C^{14} from 3-acetoxy-3,5-cholestadiene-3-C^{14}, (4-cholesten-3-one-3-C^{14} enol acetate), by Method I has been described in detail by Schwenk;[1] yield 71%, m.p. 146–148°. The preparation of cholesterol-4-C^{14} in the same manner has been reported.[10]

The preparation of cholesteryl-3-C^{14} acetate (27% yield) from 4-cholesten-3-one-3-C^{14} by a seven-step method has been reported by Havinga.[11]

The preparation of cholesteryl-4-C^{14} benzoate from 4-cholesten-3-one-4-C^{14} by a three-step process has been described by Hunziker.[12] 4-Cholesten-3-one-4-C^{14} enol acetate, prepared from 4-cholesten-3-one-4-C^{14} with acetic anhydride and acetyl chloride[4] (yield 93%), was reduced to cholesterol-4-C^{14} by sodium borohydride in methanol-ether and puri-

fied by chromatography on "non-activated" aluminum oxide with petroleum ether (yield 72%); the by-product epicholesterol-4-C^{14} was recovered, m.p. 140–141° from methanol. The benzoate ester was prepared by the action of benzoyl chloride in pyridine; yield 94%, m.p. 146–148°, $[\alpha]_D^{26}$ –16.5° ± 1° (*c* 0.842, $CHCl_3$).

[1]E. Schwenk, M. Gut and J. Belisle, Arch. Biochem. Biophys., *31*, 456 (1951).

[2]V. Grignard and H. Blanchon, Roczniiki Chem., *9*, 547 (1929); through Chem. Abstracts, *24*, 1342 (1930).

[3]H. J. Hagemeyer, Jr. and D. C. Hull, Ind. Eng. Chem., *41*, 2920 (1949).

[4]U. Westphal, Ber., *70*, 2128 (1937).

[5]H. McKennis, Jr. and G. W. Gaffney, J. Biol. Chem., *175*, 217 (1948).

[6]C. W. Shoppee and G. H. R. Summers, J. Chem. Soc., *1950*, 687.

[7]B. M. Tolbert, P. T. Adams, E. L. Bennett, A. M. Hughes, M. R. Kirk, R. M. Lemmon, R. M. Noller, R. Ostwald and M. Calvin, J. Am. Chem. Soc., *75*, 1867 (1953).

[8]W. G. Dauben and P. H. Payot, J. Am. Chem. Soc., *78*, 5657 (1956).

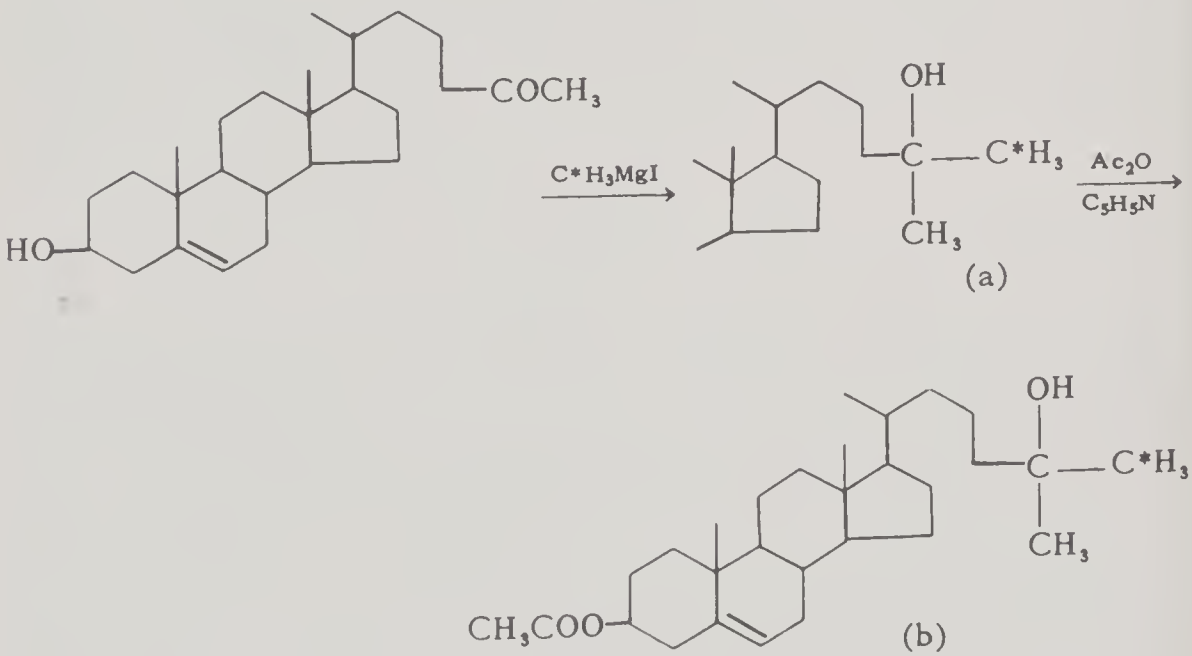
[9]F. M. Harold, M. E. Jayko and I. L. Chaikoff, J. Biol. Chem., *216*, 439 (1955).

[10]S. B. Weiss and W. Marx, J. Biol. Chem., *213*, 349 (1955).

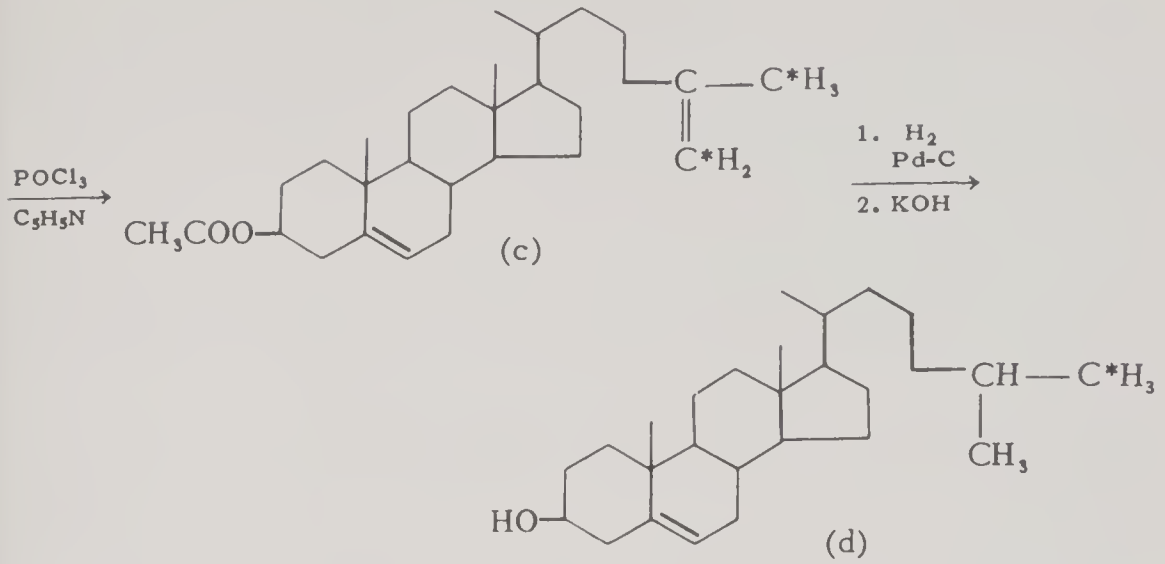
[11]E. Havinga and J. P. L. Bots, Rec. trav. chim., *73*, 393 (1954); H. Reich and A. Lardon, Helv. Chim. Acta, *29*, 671 (1946).

[12]F. Hunziker, Helv. Chim. Acta, *38*, 917 (1955).

CHOLESTEROL-26-C^{14}



W. G. Dauben and H. L. Bradlow, J. Am. Chem. Soc., *72*, 4248 (1950).



A. I. Ryer, W. H. Gebert and N. M. Murrill, J. Am. Chem. Soc., 72, 4247 (1950).

A. Procedure (Note 1)

(a) *5-Cholestene-3β,25-diol-26-C*^{14}, (*25-Hydroxycholesterol-26-C*^{14}). To a stirred solution of methylmagnesium-C^{14} iodide, prepared from 1.42 g. (10.0 mmoles) of methyl-C^{14} iodide, 0.240 g. (10.0 mmoles) of dry magnesium turnings and 40 ml. of absolute ether, is added dropwise a solution of 1.00 g. (2.85 mmoles) of 3β-hydroxynor-5-cholesten-25-one in 50 ml. of anhydrous thiophene-free benzene (Note 2). The mixture is refluxed for 4 hours, set aside at room temperature for 12 hours, decomposed with 25 ml. of water followed by 25 ml. of 50% acetic acid, and steam-distilled. The residue is filtered off, washed and dried; yield 1.07 g. (100%), m.p. 175–177°. Recrystallization from methanol gives an 84% yield, m.p. 177–179° (Note 3); $[\alpha]_D^{25}$-38.6°($CHCl_3$).

(b) *25-Hydroxy-5-cholesten-3β-yl-26-C*^{14} *Acetate.* A mixture of 13.5 g. (33.6 mmoles) of 25-hydroxycholesterol-26-C^{14}, 120 ml. of anhydrous pyridine and 30 ml. of acetic anhydride is heated at 60–70° for 1 hour, set aside overnight, then poured into water, and the product is collected on a filter and washed thoroughly (Note 4). A filtered solution of the product in 400 ml. of acetone is concentrated to one-half that volume, giving a yield of 12.7 g. (85.3%), m.p. 138.5–140.0°; $[\alpha]_D^{25}$-42.1°($CHCl_3$).

(c) *5, 25-Cholestadien-3β-yl-26,27-C*$^{14}_{1/2}$ *Acetate*, (*25-Dehydrocholesteryl-26,27-C*$^{14}_{1/2}$ *Acetate*) (Note 5). A mixture of 25.5 g. of 25-hydroxycholesteryl-26-C^{14} acetate, 750 ml. of anhydrous pyridine and 25.5 ml. of freshly distilled phosphorus oxychloride is refluxed for 30 minutes. The mixture is cooled to 20° and poured into ice-water, and the solid is collected, washed with water and dried to give 24 g. of crude product. The

solid is dissolved in hot benzene, treated with 20 g. of decolorizing charcoal and a like amount of Adsorptive Magnesia Powder (Note 6), then filtered through a bed of Celite. The filtrate is evaporated, and the solvent is replaced by methanol through distillation. The solution is concentrated to a thin slurry and set aside to crystallize at 5°; yield 21.5 g., m.p. 91.8–93.0° (Note 7).

(d) *Cholesterol-26-C*^{14} (Note 8). A solution of 5.0 g. of 25-dehydrocholesteryl-26,27-$C^{14}_{1/2}$ acetate in 500 ml. of absolute ethanol is hydrogenated over 1.25 g. of 10% palladium-on-charcoal at atmospheric pressure and room temperature. The reaction is stopped when 340 cc. of hydrogen has reacted (40 minutes). The solution is filtered, concentrated to 60 ml. and refluxed for 2 hours in the presence of 2.5 g. of potassium hydroxide. The solution is poured into water, and the product is collected and dried; yield 4.8 g., m.p. 142.4–145.2°. Recrystallization from methanol and from acetone gives 3.0 g. of pearly leaflets, m.p. 149.0–149.4° (Note 9); $[\alpha]_D^{23}$-38.6° (2% in $CHCl_3$).

B. Notes

1. Similar approaches to the preparation of cholesterol-26-C^{14} are indicated by Ryer (over 50% yield) and by Dauben (28.5% yield). The procedures were developed for isotopic labeling, but were reported in the nonisotopic form.

2. The starting material may conveniently be prepared by hydrolysis of the corresponding acetate[1] in alcoholic potassium hydroxide, and recrystallization from alcohol.

3. The pure compound melts at 181.5–182.5°. A synthesis of cholesterol-6-C^{14}, starting with a similar first step, has been proposed.[2]

4. The procedure is a modification of the method of Koechlin and Reichstein.[3] Dauben and Bradlow have prepared the compound by brominating the carbinol with phosphorus tribromide followed by dehydrobrominating by thermal cleavage in hot collidine solution. The yield is 72%, m.p. 92.5–93.5°. Dehydrohalogenation also is observed upon vacuum sublimation.

5. The diacetate is formed when the diol is refluxed with acetic anhydride containing a trace of pyridine.

6. No. 2642, Westvaco Chlorine Products Corp., Newark, Calif.

7. The pure compound melts at 93.5–94.0°; $[\alpha]_D^{20}$-43.6° (2% in $CHCl_3$).

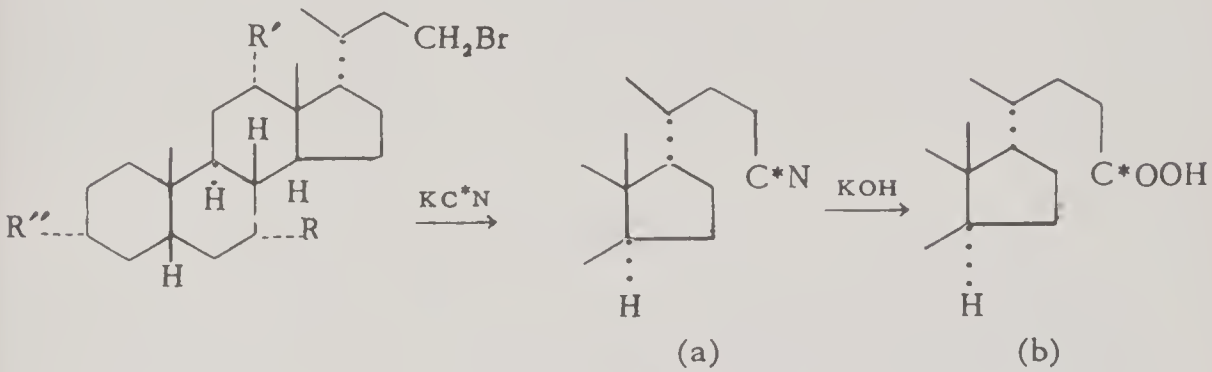
8. Dauben and Bradlow have given directions for converting the 25-dehydrocholesteryl acetate by the sequence: 25-dehydrocholesterol-26-C^{14} (91.5% yield), 25-dehydroisocholesteryl-26,27-$C^{14}_{1/2}$ methyl ether, isocholesteryl-26-C^{14} methyl ether (61% yield, m.p. 73.0–74.5°) and cho-

lesterol-26-C^{14} (87% yield), m.p. 145.0–146.0°. The over-all yield is 28.5% from methyl iodide.

9. The infrared spectrum and mixed melting point are identical with those of an authentic specimen. The yield is better than 50% based on methyl iodide.

[1]L. Ruzicka and W. H. Fisher, Helv. Chim. Acta, *20*, 1291 (1937).
[2]F. N. Hayes, Nucleonics, 7 (4), 52 (1950).
[3]B. Koechlin and T. Reichstein, Helv. Chim. Acta, *27*, 549 (1944).

LITHOCHOLIC-24-C^{14} ACID



S. Bergström, M. Rottenberg and J. Voltz, Acta Chem. Scand., 7, 481 (1953).

A. Procedure

(a) *Lithocholanonitrile-24-C^{14}*. A solution of 0.328 g. (0.8 mmole) of 23-bromo-3α-hydroxynorcholane (R = R′ = H, R″ = OH), m.p. 130–131°, (Note 1) and 0.026 g. (0.4 mmole) of potassium cyanide-C^{14} in 35 ml. of absolute ethanol and 3 ml. of water is refluxed for 48 hours (Note 2).

(b) *Lithocholic-24-C^{14} Acid, (3α-Hydroxycholanic-24-C^{14} Acid)*. To the solution of the nitrile is added 3 ml. of 30% aqueous potassium hydroxide, and refluxing is continued for 48 hours. The nonacidic materials are removed by extraction of the alkaline solution. The yield of product is 80–90% based on cyanide; m.p. 180–181° after crystallization from aqueous acetone (Note 3).

By the same procedure, from the appropriate bromides, are prepared the following bile acids:

(c) Deoxycholic-24-C^{14} acid, (R = H, R′ = R″ = OH).
(d) Cholic-24-C^{14} acid, (R = R′ = R″ = OH).
(e) Chenodeoxycholic-24-C^{14} acid, (R = R″ = OH, R′ = H)
(f) Cholanic-24-C^{14} acid, (R = R′ = R″ = H)

B. Notes

1. The preparation of the starting materials is described by Bergström, Rottenberg and Voltz. Deoxycholic acid is converted into lithocholic

acid, by the method of Bergström and Haslewood.[1] Lithocholic, chenodeoxycholic, deoxycholic and cholic acids are acetylated in an excess of acetic anhydride and pyridine by heating for 3 hours on a steam-bath. The silver salts are prepared by adding an alcoholic solution of the acids, neutralized with sodium hydroxide (phenolphthalein), to an alcoholic solution of silver nitrate. The norbromides are prepared by bromine degradation of the silver salt of the acetylated acids by the method described by Rottenberg.[2] The protecting acetyl group is then removed by refluxing in alcoholic hydrogen chloride solution.

2. The nitrile may be isolated by concentrating the solution; the melting point is 193–195° after recrystallization from dilute alcohol.

3. From the labeled acids, the different partially oxidized acids are easily available by existing methods.[3] The separation of bile acids by reversed-phase partition chromatography is described by Bergström and Sjövall.[4]

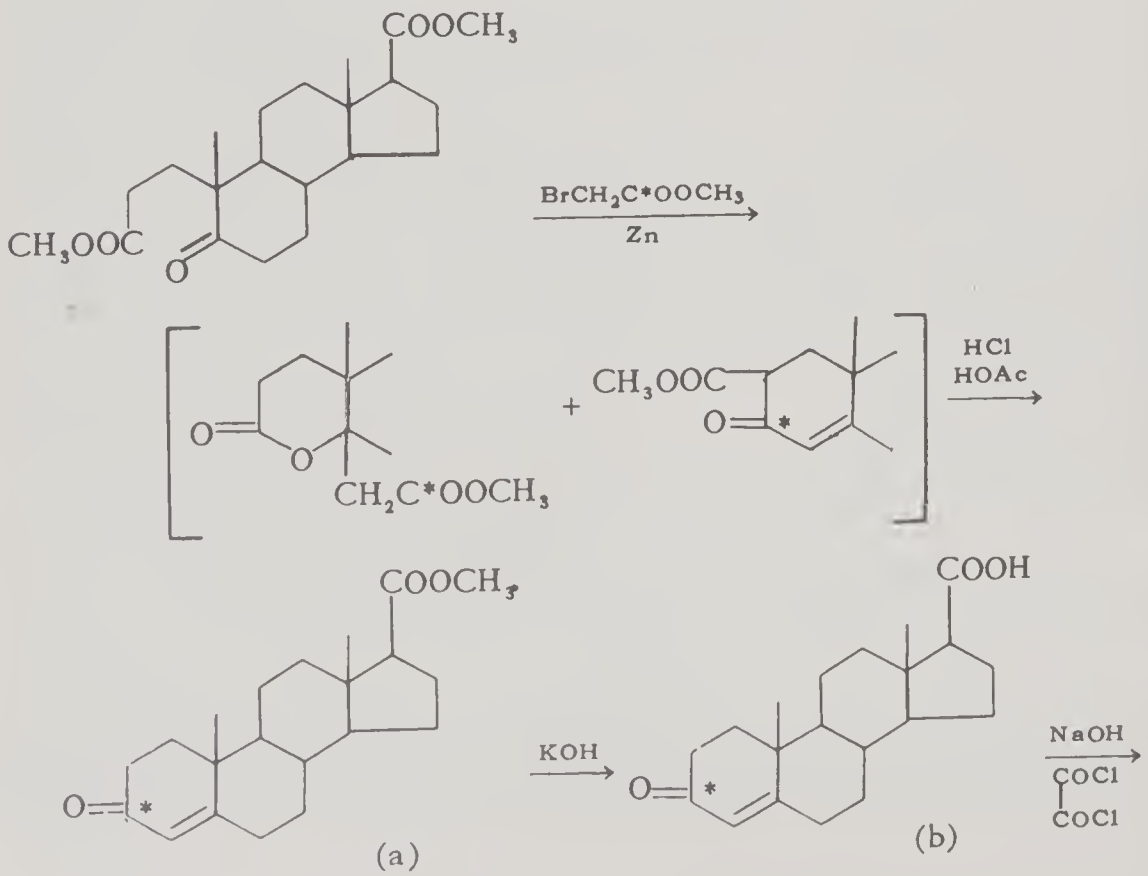
[1]S. Bergström and G. A. D. Haslewood, J. Chem. Soc., *1939*, 540.

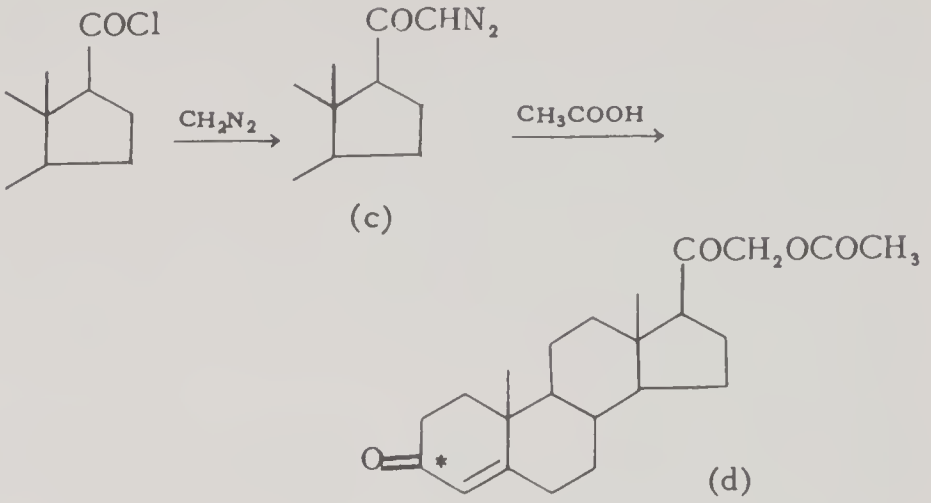
[2]M. Rottenberg, Helv. chim. Acta, *35*, 1286 (1952).

[3]L. F. Fieser and S. Rajagopalan, J. Am. Chem. Soc., *71*, 3935 (1949); *72*, 5530 (1950); *73*, 118 (1951).

[4]S. Bergström and J. Sjövall, Acta. Chem. Scand., *5*, 1267 (1951); *8*, 1611 (1954).

DEOXYCORTICOSTERONE-3-C^{14} 21-ACETATE





R. D. H. Heard and P. Ziegler, J. Am. Chem. Soc., 72, 4328 (1950).

A. Procedure

(a) *Methyl 3-Oxo-4-etiocholenate-3-C*^{14}. The methyl 3-carbomethoxydecahydro-3a,6-dimethyl-7-oxo-1*H*-benz[*e*]indene-6-propionate, (methyl 3-carbomethoxy-5-oxo-3,5-seco-4-noretiocholanoate), prepared with diazomethane from 3.17 g. of the acid (Note 1), is subjected to the Reformatsky[1] reaction (Note 2) with 2.8 g. (100% excess) of methyl bromoacetate-1-C^{14}. The ether-soluble neutral reaction products are refluxed for 5 hours in a 20% solution of concentrated hydrochloric acid in glacial acetic acid. The product is absorbed on 90 g. of alumina; fractional elution yields 0.300 g. of product, m.p. 130–132°. A like amount is recovered by recrystallizing 0.300 g. of carrier from the mother liquor.

(b) *3-Oxo-4-etiocholenic-3-C*^{14} *Acid*. The methyl 3-oxo-4-etiocholenate-3-C^{14} (0.600 g.) is refluxed for 4 hours with 6% methanolic potassium hydroxide and then acidified; yield 0.400 g.

(c) *21-Diazoprogesterone-3-C*^{14}. The yield of product is 0.240 g. (m.p. 163–170°) from 0.400 g. of the C_1^{14}-acid according to the procedure of Wilds and Shunk[2] (Note 3), which follows.

A frozen solution of 0.506 g. of 3-oxo-4-etiocholenic acid in 19 ml. of 0.091 *N* sodium hydroxide is lyophilized under reduced pressure; then the residue is dried at 110° (0.1 mm.) for 8 hours. Dry benzene (10 ml.) containing 3 drops of pyridine is added, and the salt is scraped from the walls. To the thoroughly mixed suspension, cooled in an ice-bath, is added 2 ml. of freshly redistilled oxalyl chloride (b.p. 60–60.5°). After the rapid evolution of gas, the mixture is warmed to 15° for 4 minutes; then the solvent is evaporated under reduced pressure. Dry air is admitted to the mixture, and three 1.5-ml. portions of dry benzene are added and evaporated, the temperature being kept always below 15°. A solution of the 3-oxo-4-etiocholenoyl-3-C^{14} chloride in 5 ml. of dry benzene is

filtered through a dry sintered-glass funnel into a cooled receiver and diluted with an equal volume of ether (Note 4).

The 3-oxo-4-etiocholenoyl-3-C^{14} chloride solution is added slowly to a dry (Note 5) ethereal solution of diazomethane[3,4] at $-15°$, prepared from 6 g. of 1-methyl-1-nitrosourea. The temperature of the reaction mixture is maintained at $-15°$ for 30 minutes and at $0°$ for 30 minutes; then the solvent is evaporated under reduced pressure. The residual oil is triturated with acetone to crystallize the pale yellow product; yield 0.439 g. (81%), m.p. 177–178°.[5]

(d) *Deoxycorticosterone-3-C^{14} 21-Acetate.* The yield of product is 0.065 g. from 0.125 g. of 21-diazoprogesterone-3-C^{14} by the procedure of Wilds and Shunk,[2] which follows. The product is purified by sublimation, m.p. 151–153°(Note 6).

To 10 ml. of boiling, purified acetic acid (Note 7) is slowly added 0.163 g. of 21-diazoprogesterone. Nitrogen is evolved, and heating is continued for 3 minutes. The acid is removed under reduced pressure, and the residual oil is crystallized from acetone solution, the long needles changing to powder upon drying at room temperature; yield 0.109 g., m.p. 155–157°. A second crop of 14 mg. and an additional 8 mg., obtained by molecular distillation of the filtrate at 160° (0.001 mm.), raises the total yield to 73%. Recrystallization from acetone gives a product melting at 158–159°.

B. Notes

1. The acid[6] is formed in 55% yield by opening the A ring of methyl 3-oxo-4-etiocholenate with ozone (also see progesterone-4-C^{14}, and testosterone-3-C^{14}).[7,8]

2. The detailed procedure for a somewhat similar reaction is given by Turner[8] in a preparation of 4-cholesten-3-one-3-C^{14}.

3. The α,β-unsaturated ketone is sensitive to thionyl chloride. Also see 21-diazoprogesterone-4-C^{14}.

4. The critical factor in preparing this acid chloride in maintaining a temperature below 15°.

5. A distilled solution of diazomethane is dried,[9] over potassium hydroxide for 2 hours and over sodium wire for 1 hour, for use in the Arndt-Eistert reaction.[4]

6. The decomposition of C^{14}-deoxycorticosterone during paper chromatography has been reported.[10]

7. Acetic acid is refluxed for 6 hours with potassium permanganate (5% by weight), then is distilled, and the distillate is fractionated (b.p. 117°).

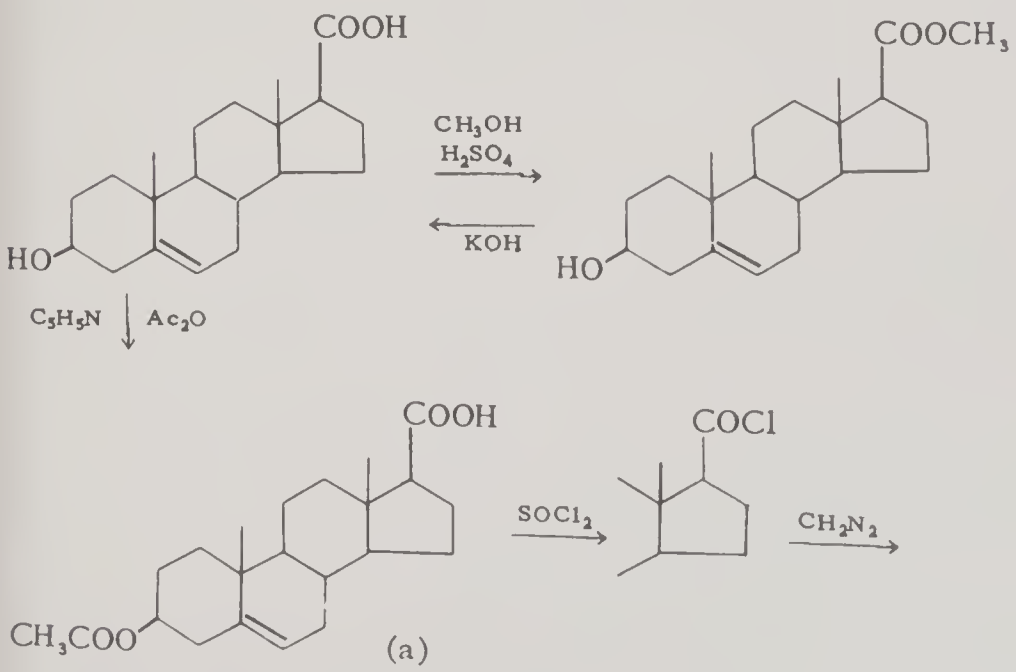
C. Other Preparations

By the same procedure from methyl bromoacetate-2-C^{14} are prepared: methyl 3-oxo-4-etiocholenate-4-C^{14}, 3-oxo-4-etiocholenic-4-C^{14} acid, 3-oxo-4-etiocholenoyl-4-C^{14} chloride, 21-diazoprogesterone-4-C^{14}, and deoxycorticosterone-4-C^{14} 21-acetate.

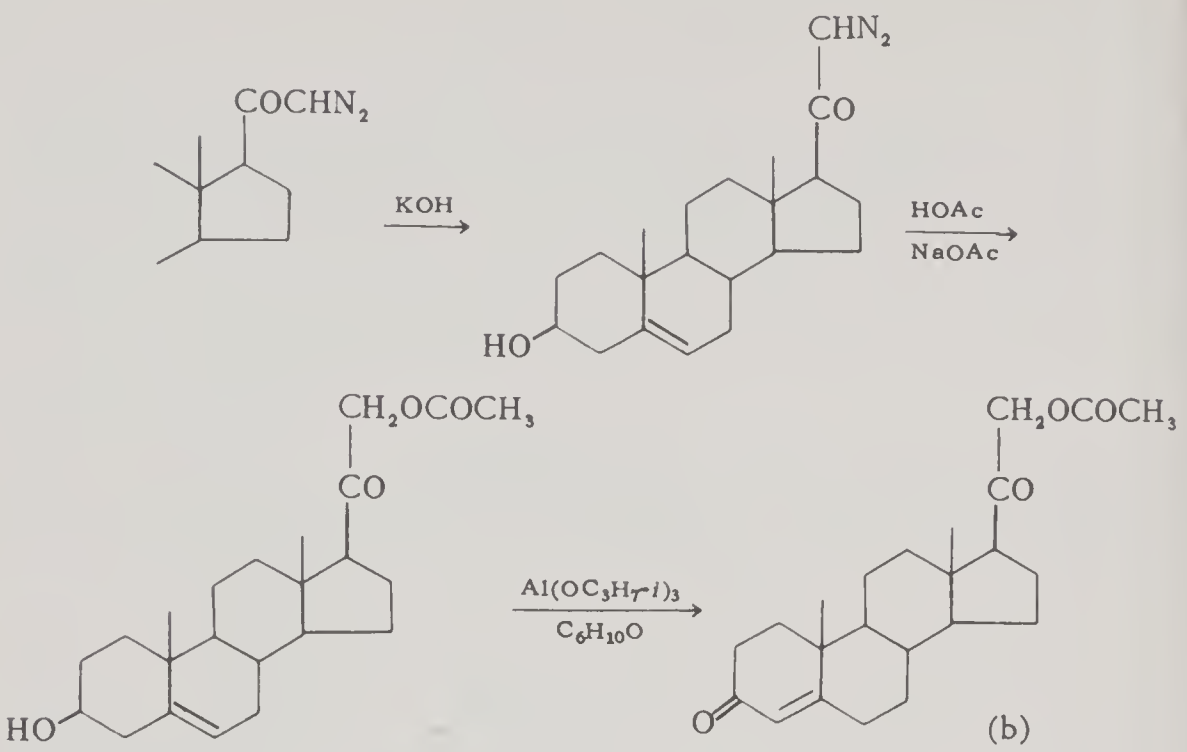
Deoxycorticosterone-21-C^{14} has been prepared,[11] presumably by treating the nonisotopic acid chloride of (b) with diazomethane-C^{14}.

[1]*Organic Reactions*, Vol. I., Wiley, New York, 1942, p. 1.
[2]A. L. Wilds and C. H. Shunk, J. Am. Chem. Soc., *70*, 2427 (1948); M. Steiger and T. Reichstein, Helv. Chim. Acta, *20*, 1164 (1937).
[3]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 165.
[4]*Organic Reactions*, Vol. I, Wiley, New York, 1942, Chapter 2.
[5]T. Reichstein and J. v. Euw, Helv. Chim. Acta, *23*, 136 (1940).
[6]T. Reichstein and H. G. Fuchs, Helv. Chim. Acta, *23*, 676 (1940).
[7]G. T. Fujimoto and J. Prager, J. Am. Chem. Soc., *75*, 3259 (1953).
[8]R. B. Turner, J. Am. Chem. Soc., *72*, 579 (1950).
[9]L. F. Fieser and R. B. Turner, J. Am. Chem. Soc., *69*, 2335 (1947).
[10]K. Savard, H. W. Wotiz, P. Marcus and H. M. Lemon, J. Am. Chem. Soc., *75*, 6327 (1953).
[11]*Recent Progress in Hormone Research*, Vol. IX, Academic Press, New York, 1954, p. 402.

C^{14}-DEOXYCORTICOSTERONE 21-ACETATE



(a)



E. Schwenk, N. T. Werthessen and A. F. Colton, Arch. Biochem. Biophys., 48, 322 (1954).

A. Procedure (Note 1)

(a) *C^{14}-3β-Acetoxy-5-etiocholenic Acid.* To a cooled, stirred mixture of 16 g. of very crude C^{14}-3β-hydroxy-5-etiocholenic acid (Note 2) and 160 ml. of methanol is added 2.4 ml. of concentrated sulfuric acid in excess of that required to give a blue reaction to Congo indicator. The stirred mixture is refluxed for 3 hours, then cooled to 15° and made basic by the addition of 18.3 ml. of 6.7 *N* methanolic potassium hydroxide in excess of that required to turn phenolphthalein paper (22 ml.). The cooled mixture is stirred for 2 hours, stored overnight at room temperature and extracted with ether. The residue (2.6 g.) obtained by evaporation is dissolved in benzene and placed on a column of silica gel (40 g.). Evaporation of a 5% ether in benzene eluate yields 0.278 g. of methyl C^{14}-3β-hydroxy-5-etiocholenate, m.p. 166–170° (Note 3).

A mixture of the ester and 0.472 g. of potassium hydroxide in 4 ml. of methanol is refluxed for 5 hours, then cooled, made just acid to litmus with 0.5 ml. of acetic acid in 0.4 ml. of methanol and stored overnight in the cold. The solid is collected, washed with cold methanol and stirred with 15 ml. of 1.26 *N* hydrochloric acid at 45° for 3 hours. The crude C^{14}-3β-hydroxy-5-etiocholenic acid is collected, washed with warm water and dried; yield 0.224 g. (Note 3).

The acid is acetylated by standing overnight with 2 ml. of acetic anhydride and 10 ml. of pyridine, then poured onto ice and 10 ml. of hydrochloric acid. The product is extracted into ether solution, which is

evaporated and dried under vacuum over phosphorus pentoxide; yield 0.2465 g., m.p. 209–230° (Note 3).

(b) *C^{14}-Deoxycorticosterone 21-Acetate, (C^{14}-21-Acetoxyprogesterone)* (Note 4). The acid is dissolved in 2 ml. of purified thionyl chloride[1] at −13° and stored for 3 hours at −8 to −12° with occasional mixing. The temperature is raised slowly to 0° during 1 hour, then to 30° during 30 minutes. The mixture is evaporated to dryness under reduced pressure below 60°, and the residue is stored overnight over phosphorus pentoxide (Note 5). To a solution of the C^{14}-3β-acetoxy-5-etiocholenoyl chloride in 1.9 ml. of purified dichloromethane (Note 6) is added a dichloromethane solution of diazomethane,[2] freshly prepared from 1.3 g. of 1-nitroso-1-methylurea, and the mixture is stored overnight. The excess diazomethane is destroyed by the addition of alumina, and the solution is filtered through a column of sodium chloride. The solution of C^{14}-3β-acetoxy-21-diazo-5-pregnen-20-one (Note 7) is mixed with an ice-cold solution of 0.5 g. of potassium hydroxide in 25 ml. of methanol, and the mixture is stored in the cold overnight (Note 8). The mixture is diluted with water, and the C^{14}-21-diazo-3β-hydroxy-5-pregnen-20-one is collected and washed with water; yield 0.2233 g. (Note 9). The compound is heated for 45 minutes at 90° with a mixture of 0.7 ml. of glacial acetic acid and 0.007 g. of sodium acetate; then the mixture is stored overnight in the cold. The crude C^{14}-21-acetoxy-3β-hydroxy-5-pregnen-20-one is isolated by ether extraction and dried under vacuum over phosphorus pentoxide; yield 0.2141 g. (Note 10).

The crude C^{14}-deoxycorticosterone is prepared by the Oppenauer oxidation as described for C^{14}-progesterone; yield 0.1980 g. Purification is accomplished by chromatography in benzene on 40 g. of silica gel, and elution with 15% ether in benzene; yield 0.0452 g. (0.12%), m.p. 150–155°, $[\alpha]_D^{26}$ + 179° (alcohol) (Note 11).

B. Notes

1. All C^{14}-compounds are randomly labeled.

2. The crude acid mixture from the oxidation of 40 g. of C^{14}-cholesterol (see C^{14}-testosterone, Note 6) probably also contains[3] C^{14}-3β-hydroxy-5-cholenic acid (m.p. 242°), C^{14}-3β-hydroxy-5-bisnorcholenic acid (m.p. 293°), and C^{14}-bilianic acid (m.p. 251°).

3. 3β-Hydroxy-5-etiocholenic acid, crystallized from dioxane, melts at 280–281° (cor.) with decomposition. The methyl ester, crystallized from methanol, melts at 179–181°. 3β-Acetoxy-5-etiocholenic acid, recrystallized from ether-pentane, melts at 241–242° (cor.).[4]

4. The procedure is a modification of that described by Steiger.[5,3] The thionyl chloride solution is protected with a calcium chloride guard tube.

5. The chloride (m.p. 165° dec.) is converted to the anhydride (m.p. 331–332° cor.) by warming in benzene.[5]

6. The solvent is purified by boiling with potassium hydroxide and washing with water.

7. 3β-Acetoxy-21-diazo-5-pregnen-20-one, m.p. 148–150°(dec.).[4]

8. The temperature is that of cold running water.

9. 21-Diazo-3β-hydroxy-5-pregnen-20-one melts at 144° (cor., dec.) after recrystallization from ether-pentane.

10. 21-Acetoxy-3β-hydroxy-5-pregnen-20-one melts at 184–185° (cor.).

11. Deoxycorticosterone acetate,[5] sublimed at 190° and 20 μ and recrystallized from acetone, melts at 157–159° (cor.); $[\alpha]_D^{19}$ 177± 4° (*c* 1, C_2H_5OH).

[1]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 570.

[2]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 165.

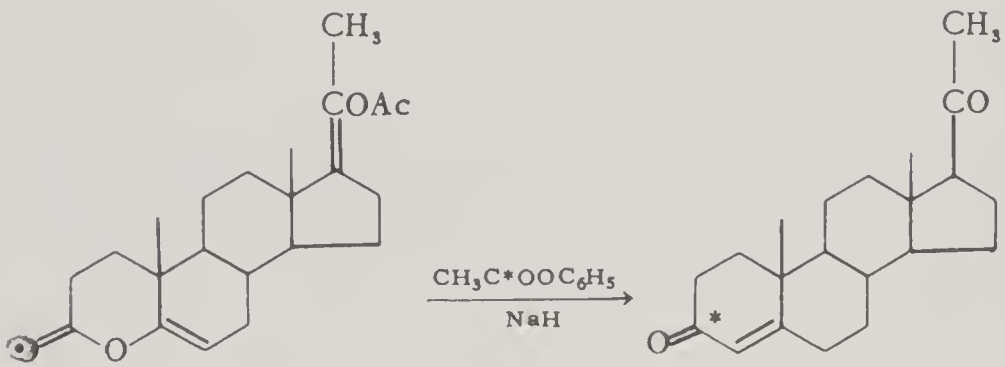
[3]L. F. Fieser and M. Fieser, *Natural Products Related to Phenanthrene*, Reinhold, New York, 1949, pp. 364 and 439.

[4]M. Steiger and T. Reichstein, Helv. Chim. Acta, *20*, 1040 (1937).

[5]M. Steiger and T. Reichstein, Helv. Chim. Acta, *20*, 1164 (1937).

PROGESTERONE-3-C^{14}

METHOD I



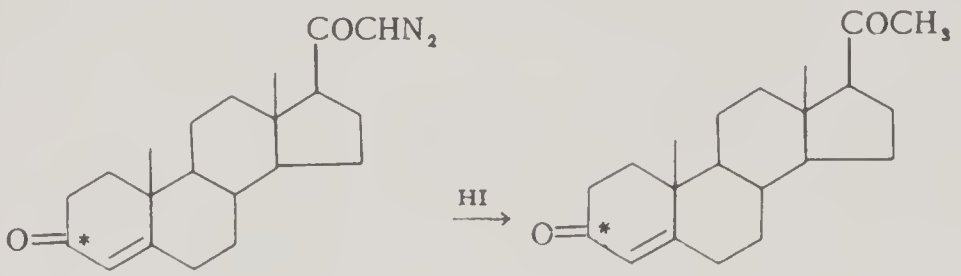
M. Gut, Helv. Chim. Acta, 36, 906 (1953).

A. Procedure (Note 1)

An anhydrous mixture of 0.500 g. of 3-(1-acetoxyethylidene)-2,3,3a,4,5,5a,6,9,9a,9b-decahydro-7-hydroxy-3a,6-dimethyl-1*H*-benz[*e*]indene-6-propionic acid δ-lactone, (4-oxa-3-oxo-5, 17-pregnadien-20-yl acetate), or 20-acetoxy-5-hydroxy-3,5-seco-4-nor-5,17-pregnadien-3-oic acid 3,5-lactone (Note 2), 0.180 g. of sodium hydride, 0.230 g. of phenyl acetate-1-C^{14} and 7 ml. of benzene is stirred under nitrogen for 48 hours. The mixture is cooled, acidified with 2 *N* hydrochloric acid and extracted with ether, and the extract is washed with water, dried over sodium sulfate and evaporated under reduced pressure. The residue is mixed

with 15 ml. of glacial acetic acid and 1.5 ml. of concentrated hydrochloric acid, then stored at 35° for 3 days under nitrogen. The mixture is evaporated under reduced pressure at 20°, and the residue is dissolved in ether. The solution is washed with 2 *N* sodium hydroxide and water, then dried over sodium sulfate and evaporated. The oily residue is purified by chromatography on a column of silica gel and elution with benzene-ether (6:1); yield 0.134 g. (25%), m.p. 129–131° (Note 3).

METHOD II



R. D. H. Heard and P. Ziegler, J. Am. Chem. Soc., 72, 4328 (1950).

A. Procedure

21-Diazoprogesterone-3-C^{14} (0.100 g.) is converted to progesterone-3-C^{14} in a yield of 0.070 g. (m.p. 122–125°), by the general reaction of Wolfrom and Brown,[1] which follows.

To a chloroform solution (15 ml.) of the diazomethyl ketone (2 g.) is added 4 ml. of 47% hydriodic acid. The mixture is shaken carefully as nitrogen is evolved, iodine is liberated, and considerable heat is generated. The reaction is completed by shaking for a few minutes; then the mixture is diluted with water, and the chloroform layer is separated, and washed with water, dilute aqueous sodium thiosulfate, and water again. The solvent is evaporated and the product is crystallized. Also see progesterone-4-C^{14}, Method II.

B. Notes

1. The method is that of Turner for labeling the steroid A ring; see testosterone-3-C^{14} and 4-cholesten-3-one-3-C^{14}.

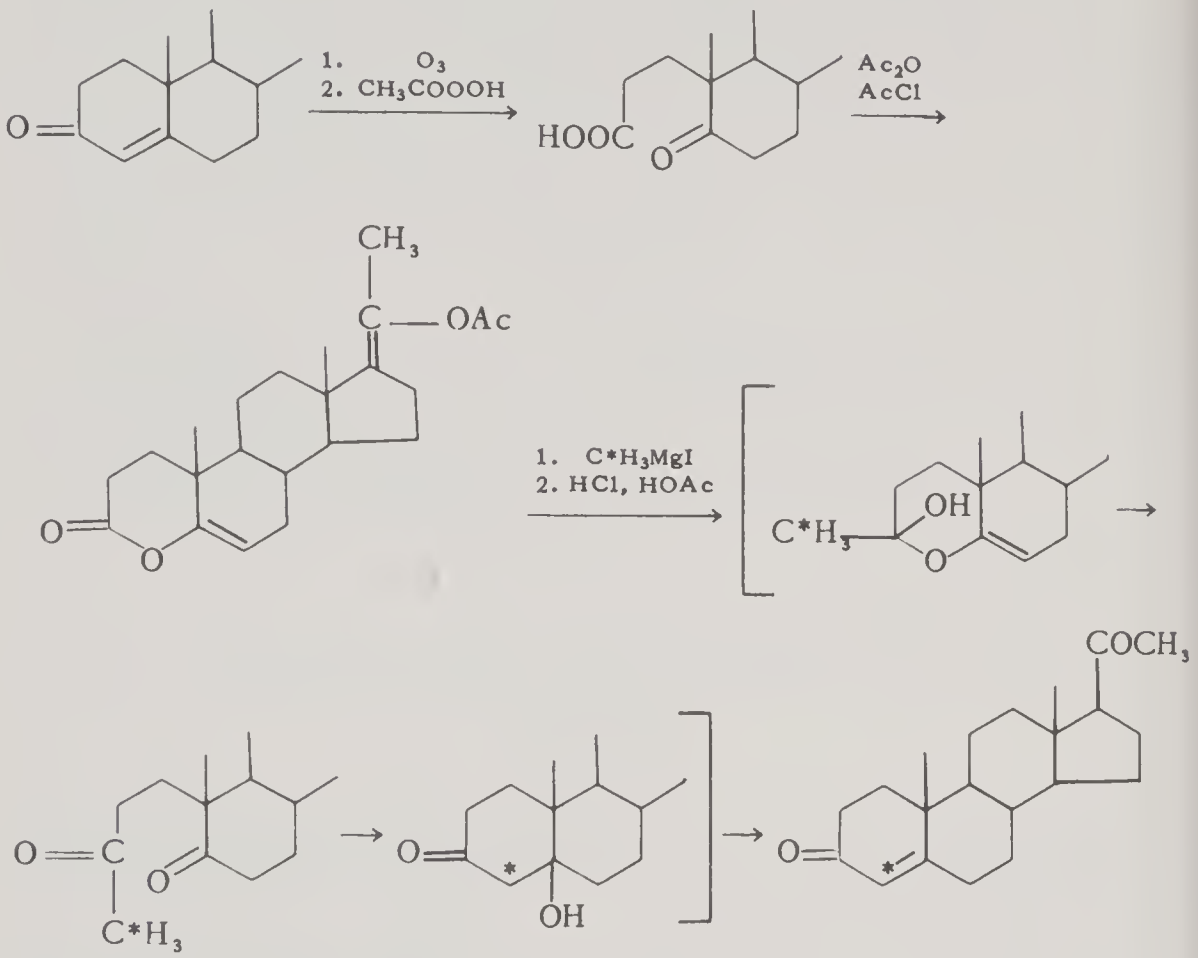
2. The preparation of the starting material (m.p. 170–180°) is described by both Gut and Fujimoto (see progesterone-4-C^{14}, Method I, Note 1).

3. The infrared spectrum and mixed melting point agree with those of an authentic sample. The molar specific activity is 88% of that of the starting material.

[1]M. L. Wolfrom and R. L. Brown, J. Am. Chem. Soc., 65, 1516 (1943).

PROGESTERONE-4-C^{14}

METHOD I



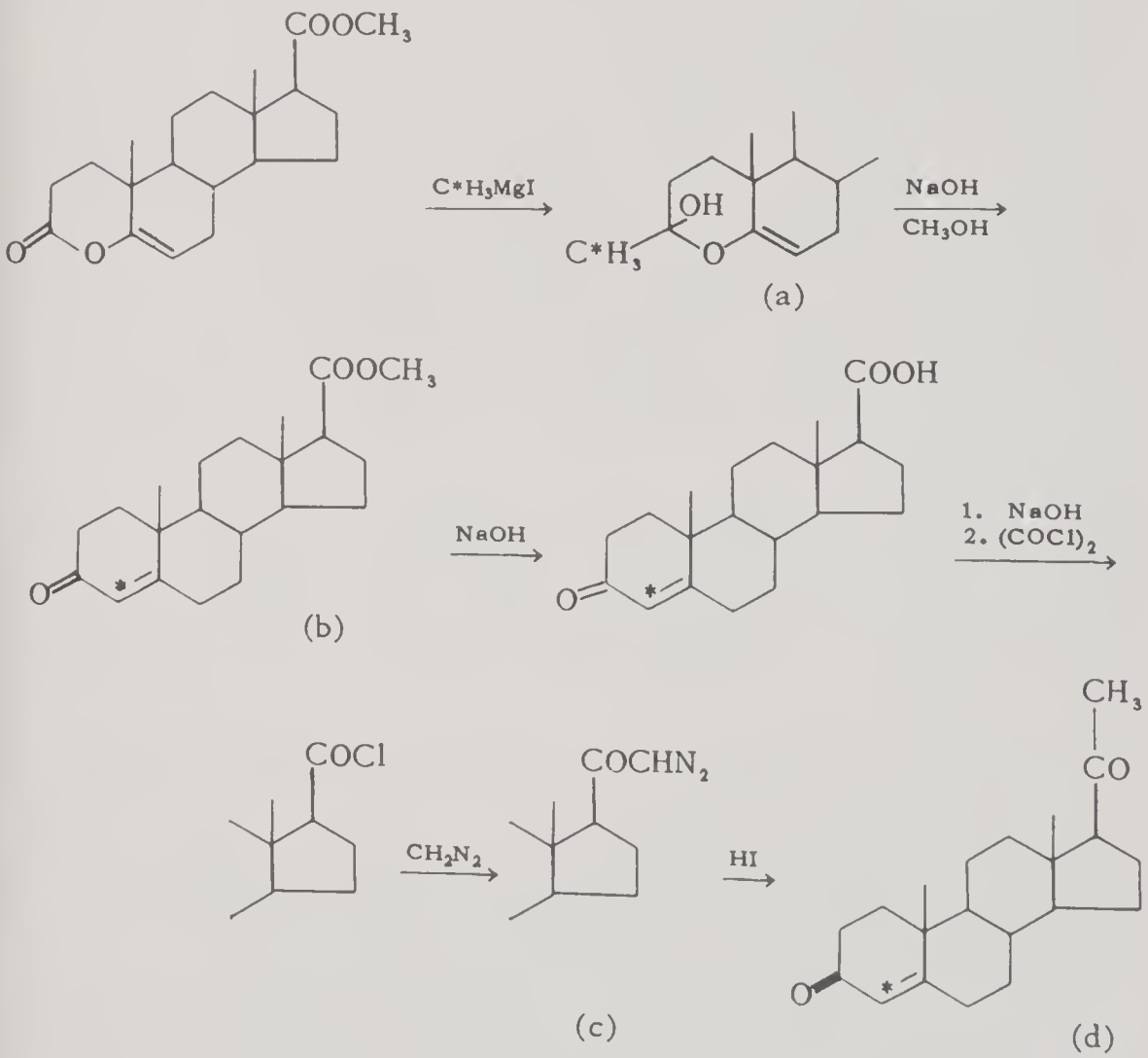
G. I. Fujimoto and J. Prager, J. Am. Chem. Soc., 75, 3259 (1953).

A. Procedure

A mixture of 0.090 g. of magnesium, 10 ml. of absolute ether, and 0.351 g. of methyl-C^{14} iodide (added *in vacuo*) is stirred at 30° for 2 hours. An atmosphere of nitrogen is introduced, and the Grignard solution is added dropwise to a stirred solution of 0.951 g. of 3-(1-acetoxyethylidene)-2,3,3a,4,5,5a,6,9,9a,9b-decahydro-7-hydroxy-3a,6-dimethyl-1*H*-benz[*e*]indene-6-propionic acid δ-lactone (Note 1) in 100 ml. of absolute ether (Note 2). The mixture is stirred for 30 minutes, then stored overnight, poured into 30 ml. of cold saturated ammonium chloride solution and stirred for 20 minutes. The hydrolysis mixture is extracted with ether, and the extracts are washed with water and evaporated. The residue is treated with 16 ml. of glacial acetic acid and 1.5 ml. of hydrochloric acid and stored at room temperature for 36 hours in an atmosphere of nitrogen to hydrolyze and to cyclize the Grignard product. The acids are removed

under vacuum, without heating, and the residual orange-colored oil is taken up in ether and washed with dilute sodium carbonate and water. The solvent is removed, and the thoroughly dried, light yellow, semi-crystalline material is divided into ten portions for purification by partition chromatography on Hyflo Supercel[1] (Note 3). The stationary phase consists of 70% aqueous methanol equilibrated with hexane, while the moving phase is hexane equilibrated with 70% methanol. Each sample aliquot, placed on the column in 0.8 ml. of stationary phase, is eluted with the moving phase and collected in 10-ml. fractions. Fractions 4–8 crystallize upon evaporation of the solvent. The product is recrystallized from acetone-hexane; yield 0.380 g., m.p. 120–122°, 46% based on methyl iodide (Note 4).

METHOD II



L. M. Thompson, C. H. Yates and A. D. Odell, J. Am. Chem. Soc., 76, 1194 (1954).

A. Procedure (Note 5)

(a) *Methyl 3-Hydroxy-3-methyl-C^{14}-4-oxa-5-etiocholenate.* Into a flask containing 0.654 g. (26.9 mmoles) of activated magnesium is vacuum-distilled and frozen 3.4 g. (23.9 mmoles) of methyl-C^{14} iodide. The mixture is diluted with 25 ml. of absolute ether under nitrogen, then stirred at room temperature for 30 minutes after the vigorous reaction ceases, and finally diluted with 75 ml. of ether.

To a stirred solution of 7.944 g. (23.9 mmoles) of methyl 4-oxa-3-oxo-5-etiocholenate (Note 6), in 70 ml. of benzene and 120 ml. of absolute ether at room temperature is added the Grignard reagent dropwise during 70 minutes. The thick mixture is stirred for 20 minutes, then diluted with 6.0 ml. of concentrated hydrochloric acid in 175 ml. of saturated salt solution. The aqueous layer is separated and extracted with ether. The combined extracts are filtered (Note 7), washed with saturated salt, 5% sodium bicarbonate, 5% sodium thiosulfate and water, and evaporated under reduced pressure; yield 8 g.

(b) *Methyl 3-Oxo-4-etiocholenate-4-C^{14}.* The crude carbinol is rearranged by stirring at room temperature for 1.5 hours in 400 ml. of methanol containing 33 ml. of aqueous 6 *N* sodium hydroxide (Note 8). The solution is concentrated under reduced pressure below 40°, and the residue is diluted with saturated salt solution and extracted with chloroform-ether (1 : 3). The extract is filtered, washed with water and evaporated, and the residue is crystallized; yield 4.257 g., m.p. 132–135.5°. An additional 0.245 g. of product is obtained by chromatography of the mother liquors on alumina (Alcoa F-20); yield 57% based on methyl iodide (Note 9).

(c) *21-Diazoprogesterone-4-C^{14}.* A mixture of 5.4026 g. of methyl 3-oxo-4-etiocholenate-4-C^{14}, 30 ml. of methanol and 13.5 ml. of 6 *N* potassium hydroxide is refluxed for 6 hours. The solvent is evaporated, and the residue is treated with dilute hydrochloric acid, yielding 4.127 g. of crude 3-oxo-4-etiocholenic-4-C^{14} acid[2] (95.7%), m.p. 225–230° (Note 10).

The sodium salt, prepared from 8.422 g. of 3-oxo-4-etiocholenic-4-C^{14} acid by employing 0.95 equivalents of methanolic sodium hydroxide, is converted to 3-oxo-4-etiocholenoyl-4-C^{14} chloride by means of oxalyl chloride in ether solution after the procedure of Wilds and Shunk, as described for 21-diazoprogesterone-3-C^{14}. The acid chloride is converted to the product with excess diazomethane; yield 4.39 g. (m.p. 178–180° dec.) and 3.76 g. (m.p. 174–176° dec.), 90.1% based on the crude acid (Note 11).

(d) *Progesterone-4-C^{14}.* The 21-diazoprogesterone-4-C^{14} is treated with 47% hydriodic acid as described by Heard and Ziegler for pro-

gesterone-3-C^{14}. The crude product (yield 87%, m.p. 120–128°) is treated with carbon and recrystallized from ether; yield 11.814 g., (37.2% based on methyl iodide), m.p. 127–131° (Note 12). The molar specific activity is 103.3% of that of the starting material.

B. Notes

1. Detailed directions are given by Fujimoto and Prager for the preparation of the starting material. Progesterone is converted to 3-acetyldodecahydro-3a,6-dimethyl-7-oxo-1*H*-benz[*e*]indene-6-propionic acid, (5, 20-dioxo-3,5-seco-4-norpregnan-3-oic acid), in 79% yield by an improvement in the ozonolysis method of Reichstein[3] (also see 4-cholesten-3-one-4-C^{14}), by decomposing the ozonide with peracetic acid. The diketo acid is then simultaneously lactonized and acetylated in acetic anhydride-acetyl chloride, forming the usable mixture of C-20 isomers 3-(1-acetoxyethylidene)-2,3,3a,4,5,5a,6,9,9a,9b-decahydro-7-hydroxy-3a,6-dimethyl-1*H*-benz[*e*]indene-6-propionic acid δ-lactone; yield 45%, m.p. 172–182°. Recrystallization from acetone raises the melting point to 181–185°. Detailed directions for this preparation are given also by Gut.[4]

2. A white precipitate forms at once.

3. A column, 20 × 2.8 cm., is packed with 24 g. of Supercel wetted with 16 ml. of stationary phase. The decomposition of C^{14}-progesterone during paper chromatography has been reported.[5]

4. The infrared spectrum, mixed melting point and biological activity are identical with those of an authentic sample. The yield agrees with that obtained for the synthesis of 4-cholesten-3-one-4-C^{14} and testosterone-4-C^{14}. A preparation using 1.0 mmole of methyl-C^{14} iodide and 0.360 g. of the enol-lactone enol-acetate yielded 0.080 g. (25% yield) of product. This low figure was attributed to the deteriorated condition of the sample of methyl-C^{14} iodide used. Hydrolysis of the side chain enol acetate does not give rise to any isoprogesterone.

The isotopic product has been purified by chromatography on silica gel and elution with benzene-ether (6 : 1).[4]

5. The procedure is a modification of the method of Fujimoto, see Method I.

6. The starting material, m.p. 168–170°, is prepared in good yield from methyl 3-oxo-4-etiocholenate by a slight modification of the method of Turner (see 4-cholesten-3-one-3-C^{14}, Note 1; and testosterone-3-C^{14}, Note 1). The use of sodium carbonate, rather than sodium hydroxide, prevents the hydrolysis of the labile 17-carbomethoxy group during the separation of neutral from acidic oxidation products. The lactonization is effected smoothly by refluxing the keto acid with acetic anhydride containing 9% anhydrous sodium acetate.

7. From two such runs is collected 1.002 g. of over-reacted[2] material, m.p. 215–230°. The optimum yield of C^{14}-carbinol requires the use of 1.0–1.1 equivalents of Grignard reagent. The product can be recrystallized, m.p. 172.5–174.5°, with prohibitive losses.

8. Prolonged heating gives 3-oxo-4-etiocholenic-4-C^{14} acid.

9. The yield in another isotopic run was 57.8%. An additional small amount of product (2.4%) may be recovered by acidifying the combined alkaline solutions, extracting the acid, esterifying with diazomethane, and resubmitting the ester to alkaline treatment.

10. Purification by recrystallization from chloroform and from ethyl acetate raises the melting point to 253–257°.

11. Another isotopic preparation gave 89.1% conversion.

12. $[\alpha]_D^{21}$ 203 ± 3° (chloroform). A final trace of color is removed by chromatography on untreated alumina (Alcoa F-20), giving an 81% recovery of pure product, m.p. 132–133.5°.

C. Other Preparations

Heard[6] has reported the preparation of progesterone-4-C^{14} from methyl bromoacetate-2-C^{14} by a synthesis involving many steps and low over-all yield (see progesterone-3-C^{14}, Method II).

[1]W. R. Butt, P. Morris, C. J. O. R. Morris and D. C. Williams, Biochem. J., *49*, 434 (1951).

[2]R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler and W. M. McLamore, J. Am. Chem. Soc., *74*, 4230 (1952).

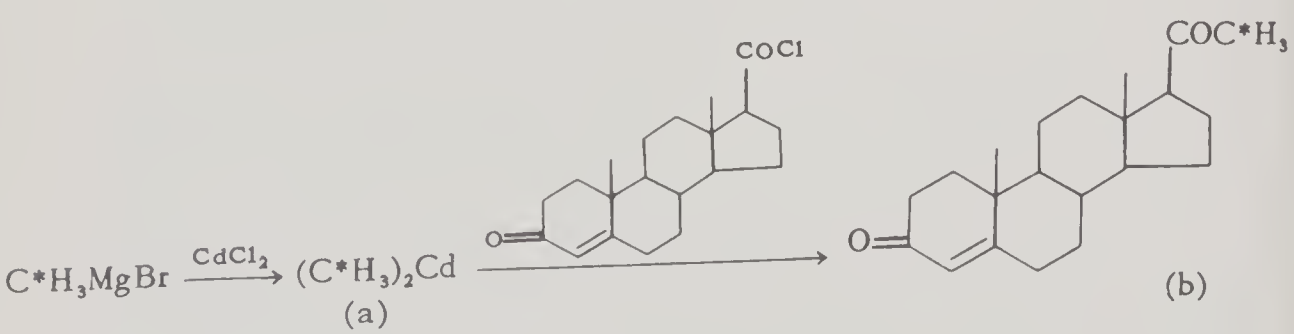
[3]T. Reichstein and H. G. Fuchs, Helv. Chim. Acta, *23*, 682 (1940).

[4]M. Gut, Helv. Chim. Acta, *36*, 906 (1953).

[5]K. Savard, H. W. Wotiz, P. Marcus and H. M. Lemon, J. Am. Chem. Soc., *75*, 6327 (1953).

[6]R. D. H. Heard and P. Ziegler, J. Am. Chem. Soc., *72*, 4328 (1950).

PROGESTERONE-21-C^{14}



B. Riegel and F. S. Prout, J. Org. Chem., *13*, 933 (1948).

A. Procedure

(a) *Dimethylcadmium-C_2^{14}*. To a frozen mixture of 0.060 g. (2.5 mmoles) of magnesium and 11 ml. of absolute ether (Note 1), contained in a two-necked 50-ml. flask equipped with a magnetic stirrer, is added 0.59 mmole of methyl-C^{14} bromide (in three portions) by vacuum technique. The mixture is warmed to room temperature, stirred for 30 minutes and frozen; then the next aliquot of methyl-C^{14} bromide is added and allowed to react as before (Note 2). An atmosphere of dry nitrogen is admitted to the system, 0.26 g. (1.4 mmoles) of powdered anhydrous cadmium chloride is added, and the mixture is stirred at room temperature for 2 hours (Note 3).

(b) *Progesterone-21-C^{14}*. To the solution of dimethylcadmium-C_2^{14} is added a solution of 3-oxo-4-etiocholenoyl chloride (Note 4) in 10 ml. of benzene. The gummy mixture is stirred for 10 hours and then refluxed for 3 hours. Dilute sulfuric acid (5 ml., 1:24) is added and heating is continued for 1 hour (Note 5). The mixture is cooled and extracted with benzene. The combined extracts are washed with water, 5% potassium carbonate solution, water and saturated sodium chloride solution, then dried with sodium sulfate, and the solvent is evaporated; yield of crude oil 0.375 g. The product is purified by chromatographic partition on 10 g. of alumina (Note 6). The chromatogram is developed with 50 ml. of benzene, and the product is eluted with 50 ml. of ethyl acetate-benzene (1:4). The crude yield is 0.290 g.; molecular distillation of this material at 0.5 μ gives three fractions: (a) 120–155°, 0.214 g., (b) 155–190°, 0.031 g. (c) 190–200°, 0.020 g. Recrystallization of fraction (a) from a mixture of 3 ml. hexane-0.5 ml. acetone yields 0.147 g. of prisms, m.p. 122.1–125.6°. As an additional 0.046 g. is recovered by reworking the other fractions and mother liquor, the total yield is 29.2% based on methanol.

B. Notes

1. The ether contains one drop of 0.1 *M* methylmagnesium bromide to assure dryness.

2. Titration[1] of a trial preparation showed the yield to be 80%. Gilman,[2] in a study of the effect of preparative conditions upon the yield of Grignard reagents, has prepared the iodide in yields of 90.1–91.7%, and the chloride in 84% yield.

3. The organometallic compound is generally used in the ether solution in which it is prepared. Gilman and Nelson[3] have reported the preparation of dimethylcadmium and the conversion to acetophenone by these methods; yield 84–95% based on methyl iodide. In general, dimethylcadmium is more satisfactory than other alkylcadmium compounds in

reactions with aroyl chlorides. Cason[4] has reported that the use of benzene and higher temperatures greatly improves the yield and removes the restriction that it is preferable for the aromatic radical to be in the cadmium reagent; also see 3-methylcholanthrene-6-C^{14}, Method I.

4. The acid chloride is prepared from 0.595 g. of sodium 3-oxo-4-etiocholenate (see 21-diazoprogesterone-3-C^{14}).

5. Heating rearranges[5] any of the physiologically inactive 17-isoprogesterone.

6. Acid-washed alumina (80–200 mesh) is activated at 250°.

C. Other Preparations

Progesterone-21-C^{14} has been prepared in a similar manner (26.2%) by MacPhillamy and Scholz.[6] 5-Pregnen-20-one-3β-yl-21-C^{14} acetate was formed in 50.6% yield, m.p. 138–144°, by condensing 3-acetoxy-5-etiocholenoyl chloride with dimethylcadmium-C_2^{14}. Saponification yielded 3β-hydroxy-5-pregnen-20-one-21-C^{14} (91.5% crude yield, m.p. 165–175°), and oxidation by the Oppenauer method yielded the product, m.p. 126–128°.

Progesterone-21-C^{14} has been prepared[7] in 0.200-g. yield from 2 mmoles of methyl-C^{14} iodide, by essentially the method described. Purification was effected by further chromatography on magnesium silicate-Celite (1:1), instead of by molecular distillation, $[\alpha]_D^{25}$ +191°. The preparation has been reported, also, by Heard[8] (25% yield).

[1]H. Gilman, P. D. Wilkinson, W. P. Fishel and C. H. Meyers, J. Am. Chem. Soc., *45*, 150 (1923).

[2]H. Gilman, E. A. Zoellner, W. M. Selby and C. Boatner, Rec. trav. chim., *54*, 584 (1935).

[3]H. Gilman and J. F. Nelson, Rec. trav. chim., *54*, 518 (1935).

[4]J. Cason, J. Am. Chem. Soc., *68*, 2078 (1946).

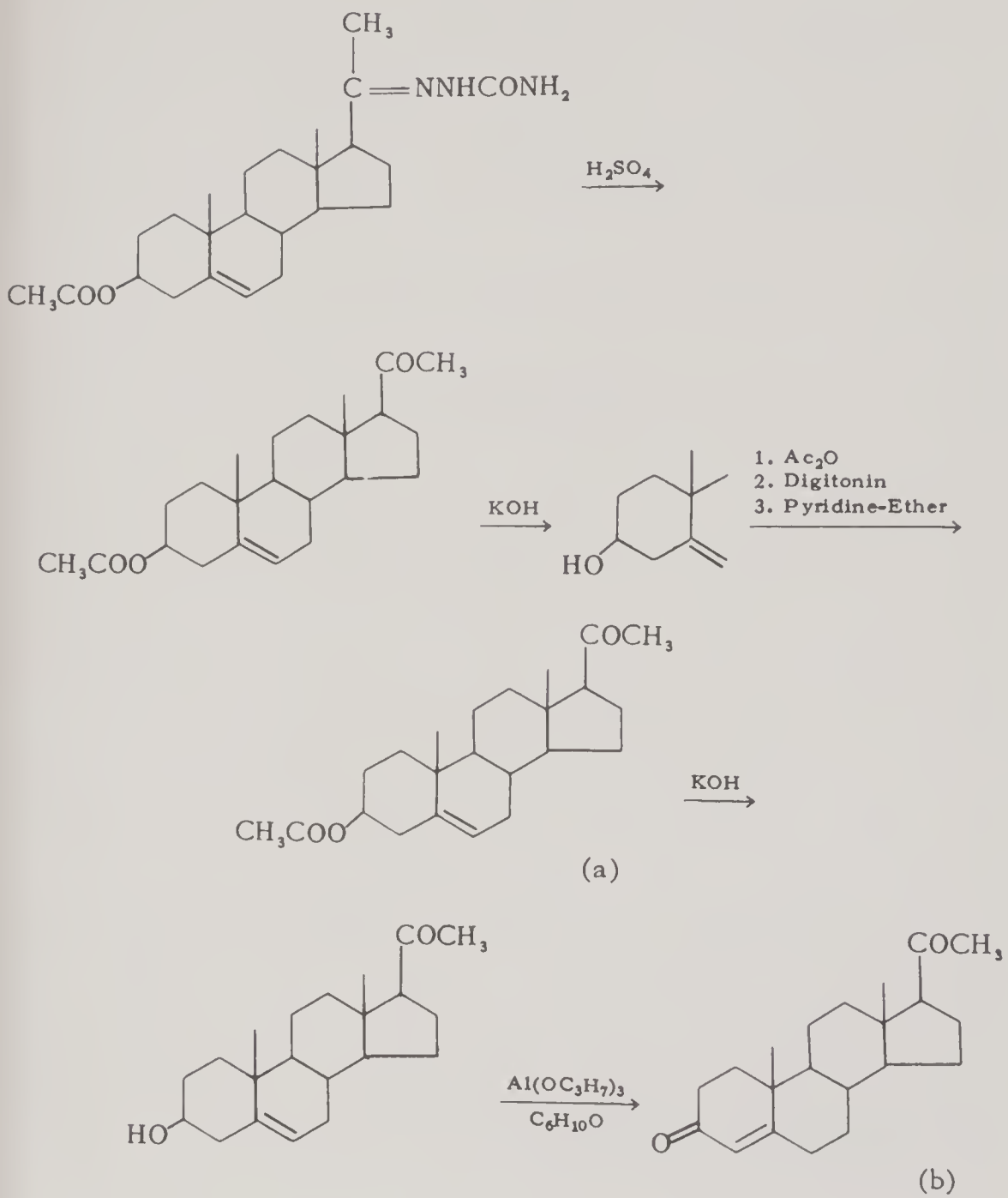
[5]A. Butenandt, J. Schmidt-Thome and H. Paul, Ber., *72*, 1112 (1939).

[6]H. B. MacPhillamy and C. R. Scholz, J. Biol. Chem., *178*, 37 (1949).

[7]H. J. Grady, W. H. Elliott, E. A. Doisy, Jr., B. C. Bocklage and E. A. Doisy, J. Biol. Chem., *195*, 755 (1952).

[8]R. D. H. Heard and C. Yates, Nucleonics, 7 (4), 53 (1950); R. D. H. Heard, C. P. Leblond, J. C. Saffran, R. J. Jamieson, G. Balla, C. Yates, L. Thompson, B. Belleau, P. Ziegler and E. Prado, Intern. Congr. Biochem., Abstrs. Communs., 1st Congr., Cambridge, Engl., *1949*, 428; Chem. Abstracts, *47*, 2192 (1953).

C^{14}-PROGESTERONE



E. Schwenk, N. T. Werthessen and A. F. Colton, Arch. Biochem. Biophys., 48, 322 (1954).

A. Procedure

(a) C^{14}-*5-Pregnen-20-one-3β-yl Acetate*, (*C^{14}-Pregnenolone Acetate*) (Note 1). The residue collected from the mother liquors from the preparation of C^{14}-3β-hydroxy-5-androsten-17-one semicarbazone acetate is

washed with 3 portions of boiling pentane and 3 portions of ether. To a solution of 5.9 g. of the residue in a small amount of methanol and 67 ml. of dioxane is added 10.5 ml. of 43% sulfuric acid. The stirred mixture is heated to 60° for 1.5 hours, poured onto ice and extracted with ether, and the extract is evaporated. To a solution of the residue in 33 ml. of methanol is added 3.5 g. of potassium hydroxide in 20 ml. of methanol. The mixture is stored overnight; then the solution, containing lactones, is extracted with ether, which is then washed with water and evaporated. A solution of the residue (1.4 g.) in 14 ml. of pyridine and 1.5 ml. of acetic anhydride is stored overnight, then poured onto a mixture of ice and 14 ml. of concentrated hydrochloric acid. The acetates are extracted into ether solution, which is washed with water and evaporated. To a solution of the residue in 14 ml. of methanol is added a hot solution of 3.8 g. of digitonin in 24 ml. of methanol, followed by 5.6 ml. of distilled water and storage of the mixture overnight. The C^{14}-5-pregnen-20-one-3β-yl acetate digitonide is collected, washed with cold methanol and ether (Note 2), then heated with a small amount of pyridine for 1 hour on a steam-bath, and the digitonin is precipitated with ether. The filtered solution is evaporated, yielding 0.106 g. of crude product, m.p. 132–144° (Note 3).

(b) C^{14}-*Progesterone* (Note 4). The crude ester is hydrolyzed by refluxing with 0.800 g. of potassium hydroxide in 70 ml. of methanol. The solution is concentrated by distilling off 45 ml., and is diluted with water. The C^{14}-3β-hydroxy-5-pregnen-20-one is extracted into ether solution, which is washed with water and evaporated to dryness. A mixture of the residue with 4 ml. of toluene and 0.8 ml. of redistilled cyclohexanone is concentrated by distilling 2 ml., then is refluxed with 0.7 ml. of 20% aluminum isopropoxide in toluene for 30 minutes, and finally diluted with water and hydrochloric acid. The crude product is extracted into ether solution, which is washed with water and steam-distilled. The residue is again extracted into ether solution, which is evaporated, and the product is dissolved in benzene and placed on a column of silica gel. The fraction eluted with 20% ether in benzene is evaporated to dryness; yield 0.0249 g., m.p. 120–123° (Note 5).

B. Notes

1. All C^{14}- compounds are randomly labeled. The procedure is an adaptation of the method of Whitman[1] for the selective precipitation of the digitonide of pregnenolone acetate.

2. The mother liquor is evaporated, and the soluble digitonide is converted to C^{14}-4-androstene-3,17-dione by the hydrolysis and oxidation

procedure described for C^{14}-progesterone; yield 0.121 g., m.p. 169–171°, $[\alpha]_D^{25}$ +196°.

3. 5-Pregnen-20-one-3β-yl acetate melts[2] at 147°.

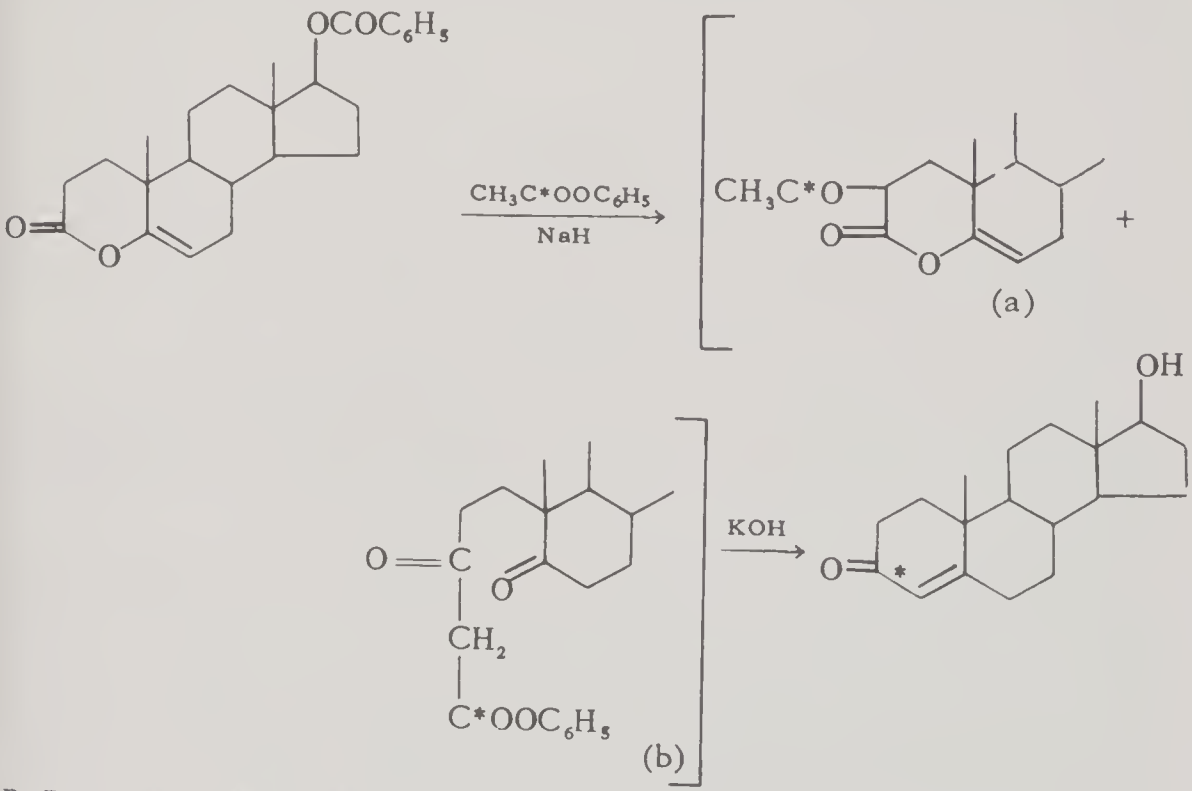
4. See the similar preparation of C^{14}-4-androstene-3,17-dione.

5. $[\alpha]_D^{26}$ +180° ($CHCl_3$). The yield is 0.07% based on 40 g. of C^{14}-cholesterol worked up in 8 batches.

[1]B. Whitman and E. Schwenk, U.S. 2,221,826; Chem. Abstracts, *35*, 1412 (1941).

[2]L. F. Fieser and M. Fieser, *Natural Products Related to Phenanthrene*, Reinhold, New York, 1949, p. 391.

TESTOSTERONE-3-C^{14}



R. B. Turner, J. Am. Chem. Soc., *72*, 579 (1950); Science, *106*, 248 (1947).

A. Procedure (Note 1)

A flask containing 0.150 g. (6.25 mmoles) of sodium hydride, 0.540 g. (3.98 mmoles) of phenyl acetate-1-C^{14}, 0.790 g. (2.00 mmoles) of 3-benzoyloxy-2,3,3a,4,5,5a,6,9,9a,9b-decahydro-7-hydroxy-3a,6-dimethyl-1*H*-benz[*e*]indene-6-propionic acid δ-lactone (Note 2) and 8 ml. of dry ben-

zene is attached to a buret system filled with dry nitrogen. The mixture is stirred at room temperature until there is no further hydrogen evolution (4 days). The mixture is acidified with dilute hydrochloric acid, and the condensation product is extracted into ether and methylene chloride, then washed, dried, and concentrated. Volatile by-products are removed at the oil pump. The residue is refluxed in an atmosphere of nitrogen for 18 hours with 100 ml. of 75% aqueous methanol containing 1.00 g. of potassium hydroxide. The solution is acidified with acetic acid and evaporated to dryness under reduced pressure; then an ethereal solution of the residue is washed with dilute alkali. The crude, neutral product is purified by chromatography on alumina (Note 3); yield 0.275 g. (48% based on enol-lactone), m.p. 148–150°. Recrystallization from ether-petroleum ether gives a pure product melting at 153–154°, and the molar specific activity is 82% of that of the starting material (Note 4).

B. Notes

1. This method is essentially the same as that employed for preparing 4-cholesten-3-one-3-C^{14}, except that cyclization is more successfully effected with dilute methanolic alkali than with acid, owing to the sensitivity of the 17-hydroxyl group to acid-catalyzed rearrangement.

2. Detailed directions for preparation of the enol-lactone (52% yield) from testosterone benzoate are given by Turner; also see testosterone-4-C^{14}, Note 1. The procedure is similar to that employed in preparing the enol-lactone for the synthesis of 4-cholesten-3-one-3-C^{14}.

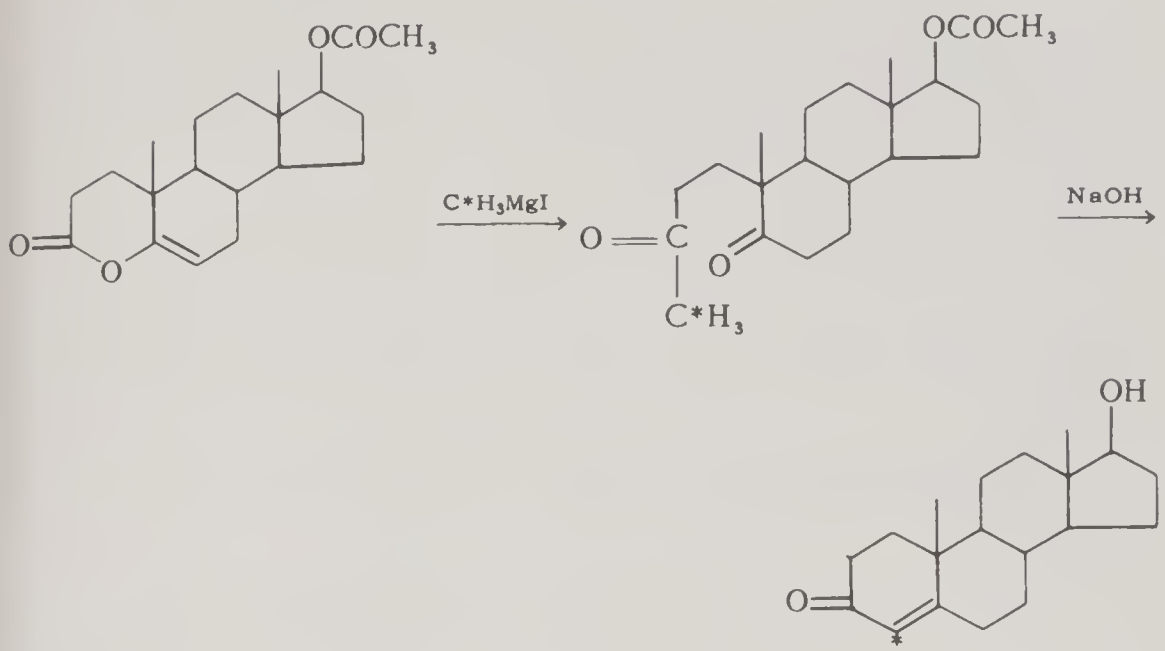
Testosterone benzoate is treated with ozone, and the ozonide is decomposed by oxidation with hydrogen peroxide and treatment of neutral fractions with periodic acid, giving a 65% yield of keto acid, m.p. 147–148° (cor.). The enol-lactone is prepared by heating the keto acid with acetyl chloride and acetic anhydride; yield 80%, m.p. 202–202.5° (cor.).

3. The effect of ultraviolet light upon testosterone-3-C^{14} and other steroids during paper chromatography has been described by Savard.[1] With the decomposition of testosterone there is a corresponding increase of polar material at the starting line and the appearance of at least five additional steroids which give positive color reactions with the Zimmermann reagent. Also see the decomposition of cholesterol-4-C^{14}.

4. The derivative testosterone-3-C^{14} acetate melts at 139–140°. The specific activity measurements indicate that 82% of the product is formed *via* intermediate (a).

[1]K. Savard, H. W. Wotiz, P. Marcus and H. M. Lemon, J. Am. Chem. Soc., *75*, 6327 (1953); K. Savard, J. Biol. Chem., *202*, 457 (1953).

TESTOSTERONE-4-C^{14}



G. I. Fugimoto, J. Am. Chem. Soc., 73, 1856 (1951).

A. Procedure

A Grignard reagent, prepared from 1 mmole of methyl-C^{14} iodide, is added dropwise to a stirred solution of 0.332 g. (1 mmole) of 3-acetoxy-2,3,3a,4,5,5a,6,9,9a,9b-decahydro-7-hydroxy-3a,6-dimethyl-1*H*-benz[*e*]indene-6-propionic acid δ-lactone (Note 1) in 3 ml. of absolute ether at 0° under nitrogen. After standing in the cold for 20 hours, the mixture is hydrolyzed and cyclized by stirring for 24 hours with a solution of 1.0 g. of sodium hydroxide in 5 ml. of water and 30 ml. of methanol. After removal of methanol, the product is washed with dilute alkali and purified by chromatography on alumina. The yield is 0.145 g. (50%) of colorless plates from acetone, m.p. 150–154°. Recrystallization from acetone gives a product, m.p. 154–155°, which is identical with an authentic sample, according to melting point and infrared absorption data.

B. Notes

1. The enol-lactone 17-acetate is used, instead of the benzoate, because of its greater ether solubility. Directions are given by Fugimoto for its preparation, which is patterned after the procedure of Turner[1] (see 4-cholesten-3-one-4-C^{14} and progesterone-4-C^{14}).

C. Other Preparations

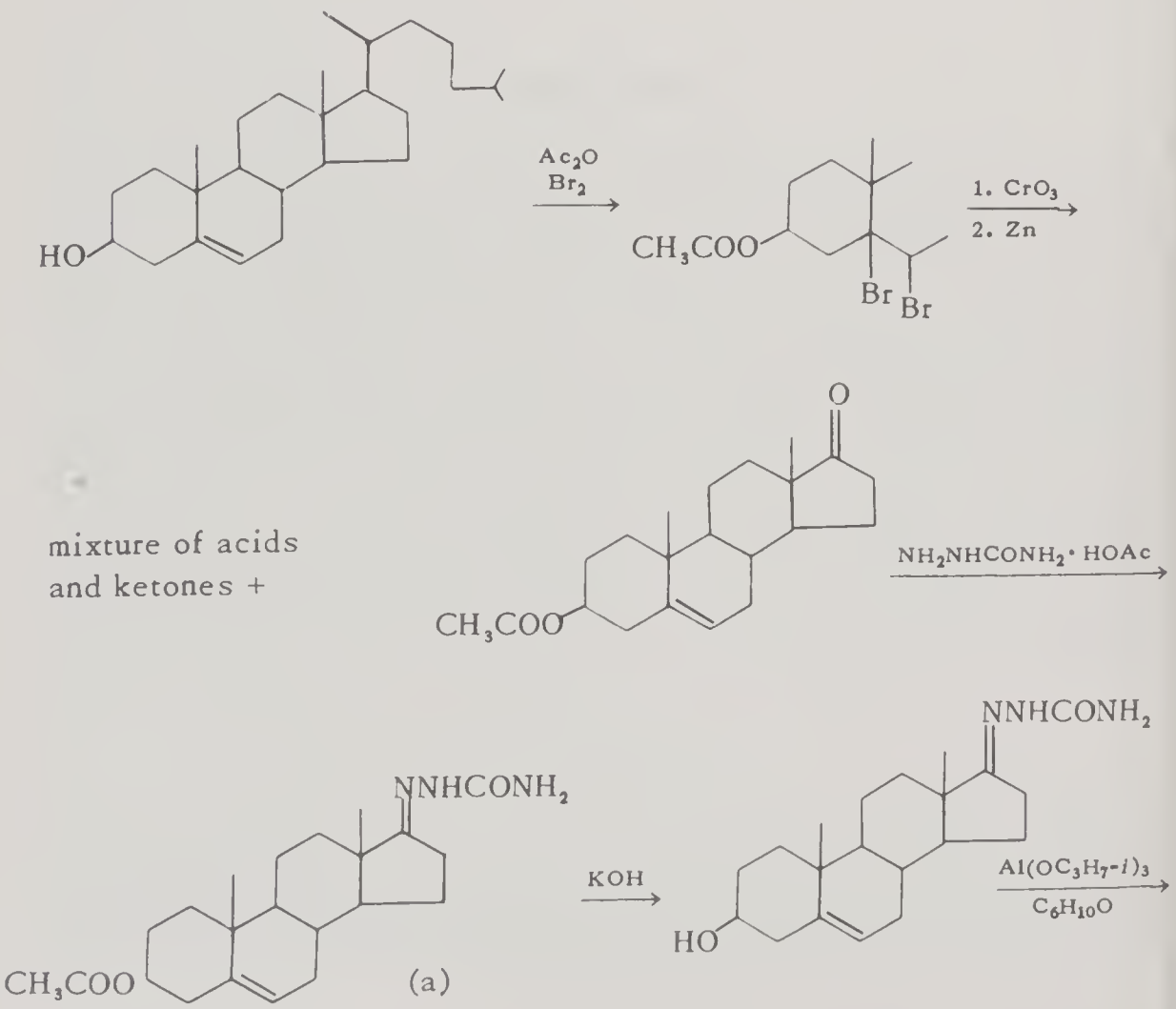
Both testosterone-4-C^{14} and -4-C^{13} have been prepared by Turner,[1] by condensing the appropriate phenyl acetate-2-C* (in excess) with the enol-lactone (benzoate), followed by simultaneous hydrolysis, decarboxylation and cyclization of the condensation product in dilute alkali; yield 48% based on the enol-lactone.

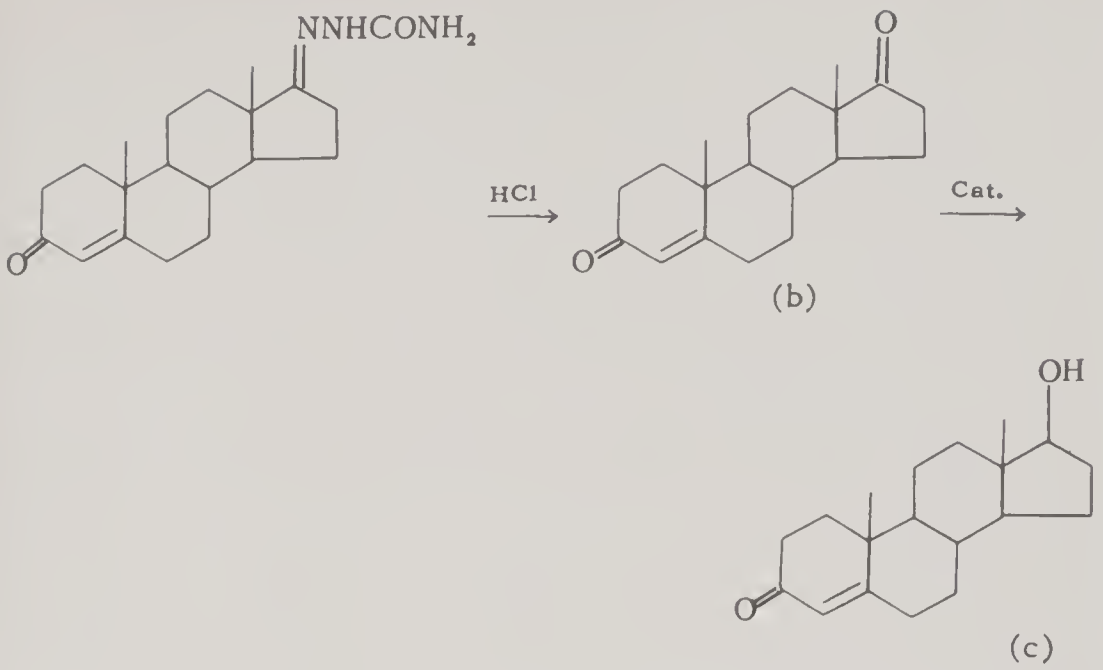
Testosterone-4-C^{14} has been prepared in 53% yield by Ashmore,[2] employing the method of Turner[1] with slight variation. The keto acid derived from ozonization of testosterone benzoate gave an enol-lactone starting material identical with that described by Turner, but melting 30° higher.

[1]R. B. Turner, J. Am. Chem. Soc., *72*, 579 (1950).

[2]J. Ashmore, W. H. Elliott, E. A. Doisy, Jr., and E. A. Doisy, J. Biol. Chem., *200*, 661 (1953).

C^{14}-TESTOSTERONE





E. Schwenk, N. T. Werthessen and A. F. Colton, Arch. Biochem. Biophys., *48*, 322 (1954).

A. Procedure (Note 1)

(a) *C^{14}-3β-Acetoxy-5-androsten-17-one Semicarbazone.* A mixture of 8.8 g. of C^{14}-cholesterol (Note 2), 4.5 ml. of acetic anhydride and 4.5 ml. of ethylene chloride is refluxed for 5 hours. The stirred mixture is diluted with 70 ml. of ethylene chloride, cooled to −12° and treated during 2 hours with a freshly prepared solution of pyridine tetrabromide (Note 3). The C^{14}-5,6-dibromo-3β-cholestanyl acetate mixture is stored overnight at a temperature below 16°, then diluted with 100 ml. of glacial acetic acid, 17.5 ml. of ethylene chloride and 30 g. of Celite. To the stirred mixture maintained below 16° (Note 4) is added a chromic acid solution over 10 hours (Note 5). The mixture is stirred overnight, while the temperature is kept below 22°. The excess chromic acid is destroyed by the dropwise addition of 5 ml. of methanol, and the solution is filtered. The filter cake is washed with a mixture of 100 ml. of acetic acid and 50 ml. of ethylene chloride, then boiled with 3 portions of ether, and the filtered extracts are concentrated and combined with the main solution. To the stirred, cooled solution is added 25 g. of zinc dust during 2 hours. The solution is filtered, the residue is washed thoroughly with ether, and the combined filtrate is diluted with water. The ether layer is separated and washed with water, 10% ice-cold potassium hydroxide (until the wash is permanently yellow), 10% sodium carbonate

and finally water (Note 6). The residue (2.5 g.), obtained by evaporating the ether, is treated in methanol solution with 15 ml. of a freshly prepared solution of semicarbazide acetate (Note 7). The stirred mixture is refluxed for 2 hours, then filtered hot, and the precipitate is washed with cold methanol and ether (Note 8); yield 0.7073 g., 7.97% (Note 9).

(b) C^{14}-4-*Androstene-3,17-dione* (Note 10). A mixture of the semicarbazone, 0.600 g. of potassium hydroxide and 50 ml. of alcohol is refluxed for 4 hours, then diluted with water and acetic acid, and cooled. The C^{14}-3β-hydroxy-5-androsten-17-one semicarbazone is collected, washed with water and dried over phosphorus pentoxide (Note 11).

A mixture of the intermediate, 18 ml. of redistilled cyclohexanone, 50 ml. of anhydrous toluene and 18 ml. of 20% aluminum isopropoxide in toluene (Note 12) is refluxed for 8 hours. The mixture is cooled, and the solid is collected, washed with toluene and methanol, and dried (Note 13). The C^{14}-4-androstene-3,17-dione 17-monosemicarbazone is hydrolyzed by refluxing for 8 hours with 18 ml. of water, 18 ml. of 90% alcohol, 6.5 ml. of concentrated hydrochloric acid and 36 ml. of benzene. The mixture is extracted with ether, and the extract is washed with water, 10% sodium carbonate and water, then evaporated; yield 0.471 g., m.p. 171.5–173°.

(c) C^{14}-*Testosterone* (Note 14). To a fermenting mixture of 40 g. of sugar, 250 ml. of tap water and 40 g. of baker's yeast is added a solution of the C^{14}-4-androstene-3,17-dione in 2 ml. of alcohol containing a few drops of Tween 20 (Note 15). The temperature is maintained at 27°, and the pH is kept at 4–5 by addition of ammonia. At 24 hours another portion of sugar water and yeast is added. At 48 hours the mixture is extracted with benzene, and the extract is washed with water, 10% potassium hydroxide and water, then filtered through anhydrous sodium sulfate and evaporated to dryness. The oily residue (0.711 g.) is dissolved in benzene and placed on a column of silica gel (60 g.). The column is eluted with 100-ml. portions of solvent: benzene yields small amounts of oil; 20% ether in benzene yields 0.0871 g. of starting material, m.p. 165–167°; 25% ether in benzene yields 0.243 g. of product, m.p. 145–148°. Purification is accomplished by recrystallization from formamide-water and from acetone-ether-pentane; yield 0.1366 g. (2.08%), m.p. 153.5–154°, $[\alpha]_D^{26}$ +111°.

B. Notes

1. All C^{14}-compounds are randomly labeled. The procedure is a modification of the methods of Wallis,[1] Ruzicka,[2] Butenandt,[3] Schwenk[4] and Fukushima,[5] and that described in ref. 6.

2. Directions are given by Schwenk for preparing[7] and purifying[8] the starting material (2 mg./g. liver) by perfusion of pig liver with sodium C^{14}-acetate. The material is isolated as the oxalic acid complex.[9]

3. Bromine (1.3 ml.) is mixed with 44 ml. of ethylene chloride and 1.0 ml. of pyridine.

4. The temperature may be maintained with a bath of circulating cold water (14°), or with a thermostat coupled to an ice-bath. Low temperature favors the formation of compounds with C-17 side chains.

5. Concentrated sulfuric acid (20 ml.) is added with cooling to a stirred solution of 23.8 g. of chromic anhydride in 30 ml. of water diluted with 110 ml. of glacial acetic acid. The filtered solution is added from a tube fitted with both a capillary and a pressure release to regulate delivery.

6. The alkaline extract is hydrolyzed overnight on a steam-bath, cooled and acidified with hydrochloric acid, and the crude C^{14}-acids are extracted into ether solution for isolation of C^{14}-3β-hydroxy-5-etiocholenic acid.

7. A mixture of 2.6 g. of semicarbazide hydrochloride, 3 g. of sodium acetate and 20 ml. of methanol is refluxed for 10 minutes and filtered.

8. The mother liquor is poured onto ice, and the precipitate is collected for the isolation of C^{14}-3β-hydroxy-5-pregnen-20-one and recovery of 0.121 g. of (b).

9. Dehydroandrosterone acetate semicarbazone may be recrystallized[1] from a mixture of benzene-alcohol with an extractor; m.p. 270° (dec.). A superior method for hydrolysis of steroid semicarbazones is described by Hershberg,[10] employing exchange.

10. The procedure is adapted from that described by Gleason.[11]

11. The semicarbazone may be crystallized[3] from hot pyridine-methanol; m.p. 262–264° (dec.).

12. Dryness is assured by distilling off 2 ml. before adding the alkoxide. For a discussion of the Oppenauer oxidation, see Wilds.[12]

13. 4-Androstene-3,17-dione 17-monosemicarbazone exhibits no definite melting point up to 350°, gradually decomposing above 275°; yield 84.5%.

14. The procedure is essentially that of Mamoli.[13] Although the yield is not as good as reported, the unreacted starting material is readily recovered.

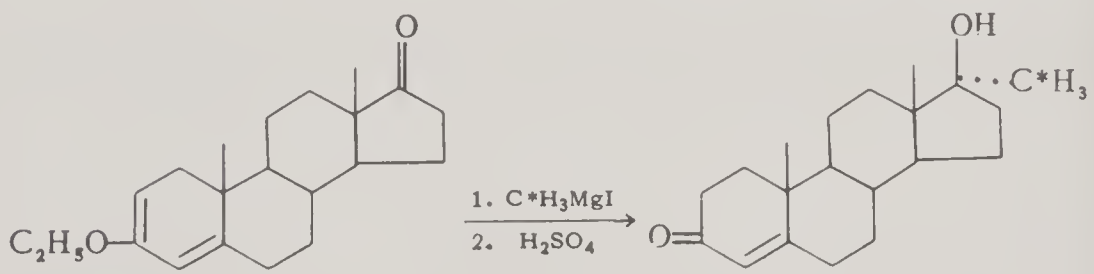
15. Polyethylene sorbitan monolaurate.

[1]E. S. Wallis and E. Fernholz, J. Am. Chem. Soc., 57, 1504 (1935).
[2]L. Ruzicka and A. Wettstein, Helv. Chim. Acta, *18*, 986 (1935).
[3]A. Butenandt, H. Dannenbaum, G. Hanisch and H. Kudszus, Hoppe Seyler's Z. physiol. Chem., 237, 57 (1935).
[4]E. Schwenk and B. Whitman, U.S. 2,244,968; C. A., 35, 5910 (1941).

[5]D. K. Fukushima and T. F. Gallagher, J. Biol. Chem., *198*, 861 (1952).
[6]*FIAT Final Report No. 996*, Office of Military Government for Germany (U.S.), 1947; L. F. Fieser and M. Fieser, *Natural Products Related to Phenanthrene*, Reinhold, New York, 1949, p. 364.
[7]N. T. Werthessen and E. Schwenk, Am. J. Physiol., *171*, 55 (1952).
[8]E. Schwenk and N. T. Werthessen, Arch. Biochem. Biophys., *40*, 334 (1952).
[9]L. Yoder, O. R. S. Sweeny and L. K. Arnold, Ind. Eng. Chem., *37*, 374 (1945).
[10]E. Hershberg, J. Org. Chem., *13*, 542 (1948).
[11]C. H. Gleason and G. W. Holden, J. Am. Chem. Soc., *72*, 1751 (1950).
[12]*Organic Reactions*, Vol. II, Wiley, New York, 1949, p. 178.
[13]L. Mamoli and A. Vercellone, Ber., *70*, 470 (1937).

17α-METHYL-C^{14}-TESTOSTERONE

METHOD I

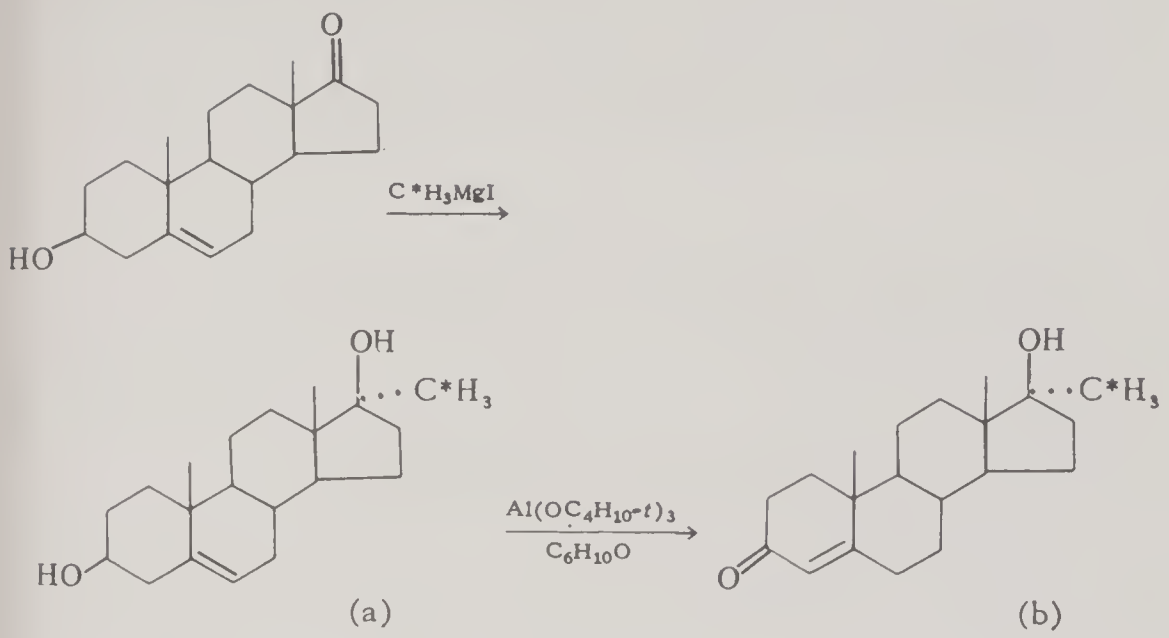


H. B. MacPhillamy and C. R. Scholz, J. Biol. Chem., *178*, 37 (1949).

A. Procedure (Note 1)

A solution of 2.96 g. of 3-ethoxy-2,4-androstadien-17-one (Note 2) in 15 ml. of anhydrous benzene is added to the Grignard solution prepared from 1.34 g. of methyl-C^{14} iodide, one equivalent of magnesium and 15 ml. of absolute ether. The mixture is refluxed for 3 hours; then the Grignard complex and the 3-enol ether of the product are hydrolyzed by stirring at room temperature for 2 hours with 25 ml. of 10% sulfuric acid. The aqueous layer is separated and extracted with two portions of ether. The combined extracts are washed with dilute sodium bicarbonate solution and water, dried and evaporated, and the residue is recrystallized from ethyl acetate. The mother liquor is evaporated, dissolved in benzene and chromatographed on alumina; the fraction eluted with 50% benzene-ether yields additional crystalline product. The combined crude product is recrystallized from ethyl acetate; yield 1.24 g. (42%), m.p. 163–164° (Note 3).

METHOD II



P. M. Hyde, W. E. Elliott, E. A. Doisy, Jr. and E. A. Doisy, J. Biol. Chem., *207*, 287 (1954); *208*, 521 (1954).

A. Procedure

(a) *17α-Methyl-C¹⁴-5-androstene-3β,17β-diol* (Note 4). To a Grignard reagent prepared from 4.55 mmoles of magnesium and 3.0 mmoles of methyl-C¹⁴ iodide, in a vacuum manifold, is added 1.06 mmoles of 3β-hydroxy-5-androsten-17-one,(dehydroepiandrosterone). The mixture is refluxed overnight, then hydrolyzed with ammonium chloride and ice, and the ether extract is washed with 5% sodium bicarbonate and water, and evaporated under reduced pressure. The crude product is purified by chromatography on magnesium silicate-Celite No. 535 (1:2), then on neutral alumina (Note 5), and recrystallized from 90% methanol; yield 0.36 mmole, m.p. 202–204° (Note 6).

(b) *17α-Methyl-C¹⁴-testosterone* (Note 7). A mixture of 10 g. of redistilled anhydrous cyclohexanone, 0.074 g. (0.243 mmole) of 17α-methyl-C¹⁴-5-androstene-3β,17β-diol and 11 ml. of anhydrous toluene is heated at 106° to remove any water by azeotropic distillation. To the refluxing solution is added 1.5 ml. of toluene containing 1.34 mmoles of aluminum *t*-butoxide (Note 8), and heating is continued for 3 hours. The mixture is poured onto ice and 0.4 ml. of glacial acetic acid, then steam-distilled to remove cyclohexanone and toluene. The aqueous solution is extracted with four 50-ml. portions of benzene, and the combined extract is washed with 30-ml. portions of 5% hydrochloric acid, water, 5% sodium bicarbon-

ate, and 3 portions of water. The extract is concentrated to 50 ml., cooled and chromatographed on a column of neutral alumina (Note 5). Following a second purification on alumina, the product is recrystallized from 80% methanol; yield 0.061 g. (83%), m.p. 164.5–165° (Note 9). The molar specific activity is the same as that of the diol.

B. Notes

1. The procedure is adapted from the method of Miescher.[1]

2. The starting material may be prepared by the method of Serini and Köster[2] for acetalization of steroid ketones with ethyl orthoformate.

3. $[\alpha]_D^{25}$ +87° in ethanol. The melting point and infrared absorption spectra agree with those of an authentic sample.

4. The procedure is an adaptation of that of Ruzicka.[3]

5. The chromatography of steroids by the method of fractional elution with solvents of increasing eluting power is described in detail by Reichstein;[4] also see 17α-methyl-C^{14}-17β-estradiol.

6. The melting point is not depressed upon admixture with an authentic sample (the 17β-methyl isomer[5] melts at 203–204° cor.). Acetylation, presumably with acetic anhydride in pyridine at room temperature,[5] forms 17α-methyl-C^{14}-5-androstene-3β,17β-diol 3-acetate, m.p. 175–176° (lit.[5] 179–180° cor., and 160–161° for the 17β-methyl isomer). Saponification yields the original product, thus establishing the configuration at C-17.

7. The procedure is a modification of that described by Oppenauer,[6] cyclohexanone being substituted for acetone as the hydrogen acceptor because of its higher oxidation potential.[7]

8. The reagent is prepared by the procedure of Wayne and Adkins.[8]

9. The melting point is not depressed upon admixture with an authentic sample, and both show log $\epsilon = 4.19$ at 240 mμ.

[1]K. Miescher, U. S. 2,386,331; Chem. Abstracts, *40*, 179 (1946).

[2]A. Serini and H. Köster, Ber., *71*, 1766 (1938).

[3]L. Ruzicka, M. W. Goldberg, and H. R. Rosenberg, Helv. Chim. Acta, *18*, 1487 (1935).

[4]T. Reichstein and C. W. Shoppee, Discussions Faraday Soc., 7, 305 (1949).

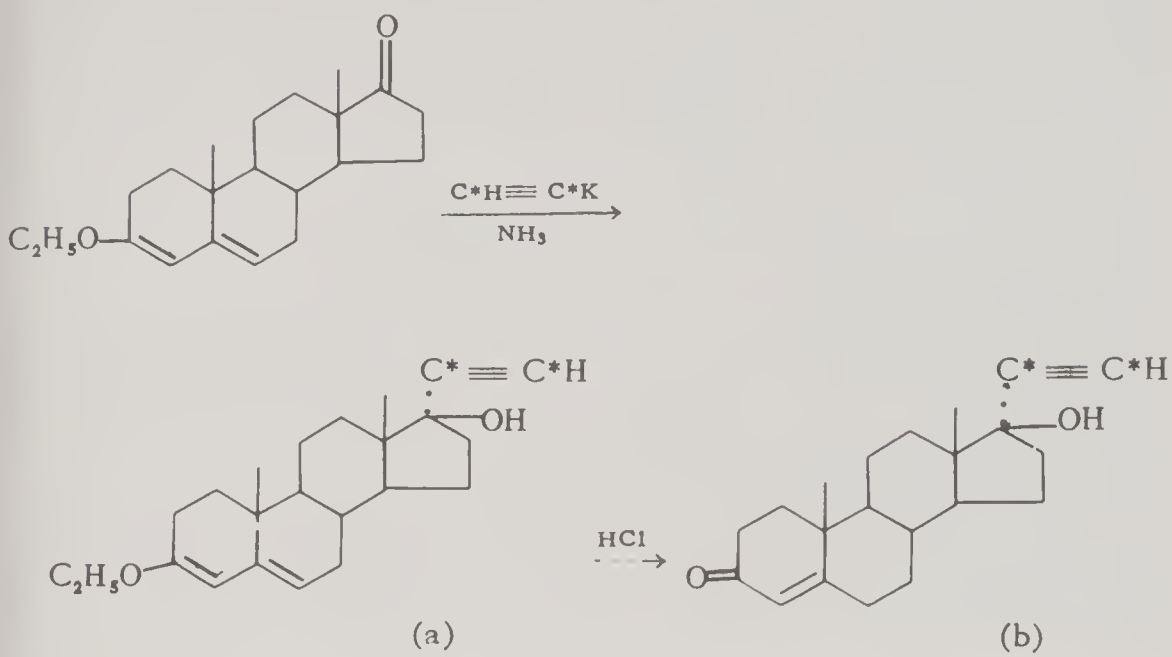
[5]K. Miescher and W. Klarer, Helv. Chim. Acta, *22*, 962 (1939).

[6]R. V. Oppenauer, Rec. trav. chim., *61*, 137 (1937).

[7]W. A. Johnson and G. E. H. Skrimshire, Chem. Ind., *1951*, 380; *Organic Reactions*, Vol. VI, Wiley, New York, 1951, Chapt. 5.

[8]*Organic Syntheses*, Vol. 21, Wiley, New York, 1941, p. 8.

17α-ETHYNYL-C_2^{14}-TESTOSTERONE



B. Riegel and Y. C. Liu, J. Org. Chem., *16*, 1610 (1951).

A. Procedure

(a) *3-Ethoxy-17α-ethynyl-C_2^{14}-3,5-androstadien-17β-ol.* The high-vacuum system employed consists of a manifold connected to a pump, a manometer, two inlets for acetylene and nitrogen, and an outlet attachment ot a reaction flask, which is equipped with an overhead induction stirrer (Note 1) and an adapter bearing a dropping funnel and side arm with stopper. All inlets and outlets are equipped with stopcocks.

Anhydrous liquid ammonia (10 ml., dried over potassium) is distilled into the reaction flask under high vacuum with Dry Ice-cooling. The system is filled with dry nitrogen, and 0.078 g. (2 mmoles) of potassium is added and dissolved by stirring. The solution is frozen with liquid nitrogen; then acetylene-C_2^{14} (0.33 mmole), which is dried by passage through a trap at $-78°$ and a tube filled with Dehydrite, is distilled into the reaction flask. The mixture is thawed, stirred and re-frozen when the formation of potassium acetylide-C_2^{14} has taken place. Purified (Note 2) tank acetylene (2.38 mmoles), dried as above, is added in portions to the reaction mixture until the stirred solution is no longer blue. The flask is cooled in a Dry Ice-bath, and dry nitrogen is admitted to the system. A solution of 0.158 g. (0.5 mmole) of 3-ethoxy-3,5-androstadien-17-one (Note 3) in 6 ml. of dry benzene and 6 ml. of absolute ether is added[1] dropwise to the stirred solution; then stirring is continued for 3 hours.

The cooling-bath is partially lowered, and the ammonia is allowed to evaporate slowly through a mercury trap into a trap containing aqueous silver nitrate solution (Note 4). After standing overnight, the reaction mixture is stirred for 30 minutes at 0° and then hydrolyzed with 15 ml. of cold, saturated ammonium chloride solution. The mixture is stirred for 15 minutes in a stream of nitrogen to transfer the remaining acetylene-C_2^{14} to the absorber. The aqueous layer is separated and extracted with ether, and the combined extracts are washed with water and saturated sodium chloride solution and dried over sodium sulfate. The product is obtained by solvent removal under reduced pressure.

(b) *17α-Ethynyl-C_2^{14}-testosterone*. The crude 3-ethoxy-17α-ethynyl-C_2^{14}-3,5-androstadien-17β-ol is dissolved in a mixture of 6 ml. of absolute alcohol, 2 ml. of 0.12 *N* hydrochloric acid[1] and 1 ml. of water (Note 5), and set aside overnight at room temperature. The mixture is concentrated to 5–6 ml. and cooled, and the product is separated, washed with cold, dilute alcohol and dried. The yield is 0.107 g. (m.p. 263.5–265°), 68.6% based on the steroid, or 13% based on acetylene (Note 6).

B. Notes

1. Diagrams of suitable vacuum-tight, magnetically-stirred reaction flasks have been published; see benzoic-C^{14} acid, (Note 2).

2. Tank acetylene is purified[2] by passage through a series of wash bottles containing, in order: a) saturated sodium bisulfite solution; b) water; c) and d) 0.2 *N* iodine in aqueous potassium iodide solution; e) and f) sodium thiosulfate solution; g) and h) alkaline sodium hydrosulfite with sodium anthraquinone β-sulfonate as indicator and oxygen carrier; i) and j) 10% aqueous potassium hydroxide solution.

3. 3-Hydroxy-5-androsten-17-one is oxidized by the Oppenauer method[3] to 4-androstene-3,17-dione, which is then etherified with orthoformic ester and hydrochloric acid by the method of Serini and Köster.[1,4]

4. The excess acetylene-C_2^{14} is recovered as silver acetylide-C_2^{14}.

5. The resultant acid concentration is 0.1%. Strong acids will catalyze the rearrangement of the C-17 tertiary carbinols.

6. Analysis indicates that the product crystallizes as a hemihydrate. A sample sublimed at 190–200° (0.5 μ) melts at 270–272°, $[\alpha]_D^{26}$ 22.7° (4 mg. in 1 ml. dioxane).

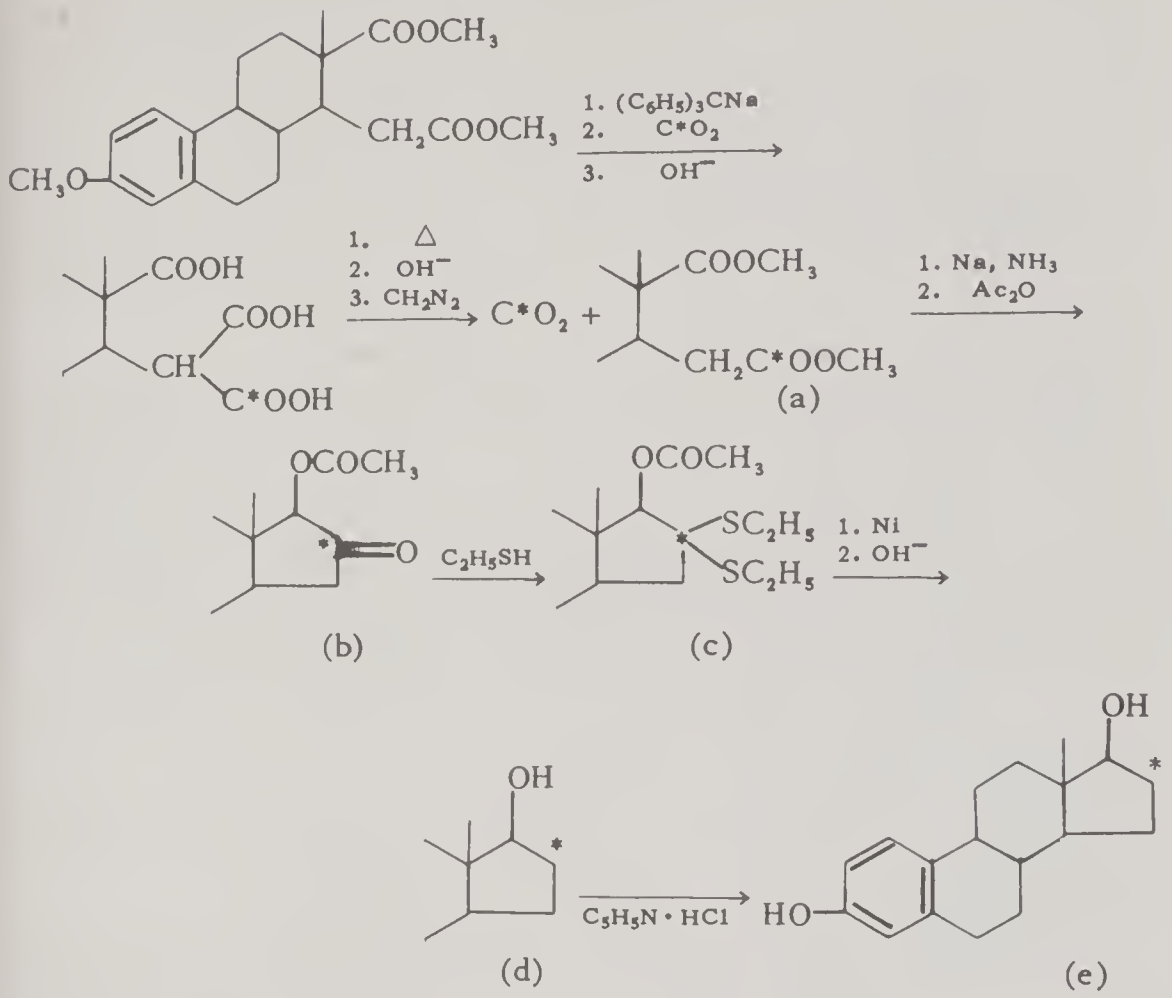
[1]H. H. Inhoffen, U. S. 2, 358, 808; Chem. Abstracts, *39*, 1738 (1945).

[2]J. B. Conn, G. B. Kistiakowsky and E. A. Smith, J. Am. Chem. Soc., *61*, 1868 (1939).

[3]A. Serini, H. Köster and L. Strassberger, U. S. 2, 379, 832; Chem. Abstracts, *39*, 5053 (1945).

[4]A. Serini and H. Köster, Ber., *71*, 1766 (1938).

17β-ESTRADIOL-16-C^{14}



M. Levitz, J. Am. Chem. Soc., 75, 5352 (1953).

A. Procedure

(a) *Methyl 2-Carbomethoxy-1,2,3,4,4a,9,10,10a-octahydro-7-methoxy-2-methyl-1-phenanthreneacetate-C^{14}, (Methyl C_1^{14}-Marrianolate Methyl Ether).* To a solution of 1.20 g. (3.33 mmoles) of methyl marrianolate methyl ether (Note 1) in 5 ml. of absolute ether, under nitrogen in a 25-ml. round-bottomed flask equipped with a magnetic stirrer, is added a solution of 3.30 mmoles of triphenylmethylsodium[1] in 14.7 ml. of ether (Note 2). The mixture is stirred for 5 minutes at room temperature, then cooled in a Dry Ice-bath, and the system is evacuated with an oil pump. The mixture is carbonated with the dry carbon-C^{14} dioxide from 0.592 g. (3 mmoles) of barium carbonate-C^{14}, with resumption of stirring after removal of the cooling-bath. The mixture is several times alternately chilled and stirred as it warms to room temperature. The ether solution is extracted with two portions of dilute acetic acid and five portions of 5% sodium carbonate solution (Note 3). The combined alkaline solutions are acidified with dilute hydrochloric acid, and the dicarboxylic acid is extracted into ether

solution, which is washed with water and evaporated to dryness. The residue is refluxed for 2 hours with a mixture of 7.5 g. of potassium hydroxide, 20 ml. of water and 12 ml. of methanol. The methanol is distilled off, 50 ml. of water is added, and the tricarboxylic acid is isolated in the same manner as above. The residual, finely divided white powder is heated in a slow stream of nitrogen (Note 4) at 180° for 1 hour; then the gas flushing is continued for 1 hour at room temperature. The yellow, glassy residue (Note 5) is refluxed for 90 minutes with a solution of 2 g. of potassium hydroxide, 10 ml. of water and 3 ml. of methanol. The acidic material is extracted into ether, in the manner described, and esterified with an excess of diazomethane in ether solution. The mixture is washed with 1% hydrochloric acid, water, and saturated sodium chloride, then evaporated. The yellow, waxy residue is dissolved in 5% ether in petroleum ether (b.p. 30–60°), then chromatographed on 10 g. of silica gel, and eluted with 25% ether in petroleum ether. The yield is 0.898 g. (83%, based on barium carbonate), m.p. 73–75° (Note 6).

(b) *17β-Acetoxy-3-methoxy-1,3,5(10)-estratrien-16-one-16-C*^{14}. The D ring is closed by submitting 0.898 g. of the methyl marrianolate methyl ether to the acyloin condensation according to the directions of Sheehan,[2] which follow. The crude 17β-hydroxy-3-methoxy-1,3,5(10)-estratrien-16 one-16-C^{14}, m.p. 166–172°, is then acetylated with acetic anhydride. The yield after one crystallization from alcohol is 0.660 g. (77%), m.p. 150.5–152° (Note 7).

To a solution of 0.8 g. of sodium in 325 ml. of anhydrous ammonia and 225 ml. of ether under purified nitrogen is slowly added a solution of 1.8 g. of methyl marrianolate methyl ether in 225 ml. of ether over a period of 1 hour. The ammonia is evaporated, and the mixture is acidified with hydrochloric acid. Purification gives 17β-hydroxy-3-methoxy-1,3,5(10)-estratrien-16-one in 60% yield (Note 8).

(c) *16,16-Bis(ethylthio)-3-methoxy-1,3,5(10)-estratrien-17β-yl-16-C*^{14}*Acetate* (Note 9). An intimate mixture of 0.660 g. of the ketone, 1.2 g. of anhydrous sodium sulfate and 0.6 g. of freshly fused zinc chloride is covered with 25 ml. of ethanethiol in a stoppered flask, then shaken until solution is complete and stored in a refrigerator for 72 hours. After removal of excess mercaptan under vacuum at room temperature, the residue is taken up alternately in *N* sodium hydroxide, and ether containing a small amount of pyridine. The solutions are mixed and the ether layer is separated, washed with three portions of water, dried over sodium sulfate and evaporated at 50°. The yield, after one crystallization from 15 ml. of alcohol, is 0.785 g. (91%), m.p. 133–135.5° (Note 10).

(d) *17β-Estradiol-16-C*^{14} *3-Methyl Ether.* The hydrogenolysis (Note 9) of the thioketal is effected by an adaptation of the procedure of Huffman[3] which follows.

To 9.21 g. of the thioketal is added about 3 g. of W-7 Raney nickel[4] in absolute ethanol, followed by 30 ml. of dioxane. The mixture is heated on a steam-bath for 7 hours under an air condenser, then stored overnight at room temperature. The mixture is filtered, concentrated under reduced pressure to a volume of 5–10 ml., diluted with several volumes of water and stored for several hours at 5°. The solid is collected, washed well with water, dried, and dissolved in acetone, and the filtered solution is evaporated to dryness.

The crude acetate is saponified[2] by refluxing for 2 hours with 50 ml. of 5% ethanolic sodium hydroxide. The mixture is concentrated to 10 ml. and diluted with 75 ml. of cold water. The product is collected, washed with water, dried, and crystallized from 0.4 ml. of warm benzene solution diluted with 2.5 ml. of hexane; yield 72%, m.p. 115–116.5° (Note 11).

(e) *17β-Estradiol-16-C*^{14}. A mixture of 0.370 g. of 17β-estradiol-16-C^{14} 3-methyl ether and 4.0 g. of pyridine hydrochloride[5] is sealed under nitrogen in a 12-mm. tube, heated to 100° and shaken to mix thoroughly, then heated for 4 hours at 180 ± 2° (see *N*-methyl-C^{14}-normorphine). The mixture is acidified with dilute hydrochloric acid, and the separated solid is extracted into ether. The ether solution is extracted with six portions of *N* sodium hydroxide; then the alkaline solution is acidified and extracted with ether. The dried ether solution is evaporated to give 0.264 g. of slightly yellow product, m.p. 176–178°. The yield, after recrystallization from 2 ml. of 95% alcohol, is 0.230 g. (64%), m.p. 177.5–178.5° (Note 12). The over-all chemical yield is 28%, while the radiochemical yield is 13% (25% based on unrecovered carbon-C^{14} dioxide).

B. Notes

1. Directions are given by Levitz for preparing the starting material. Estrone methyl ether, prepared from estrone and methyl sulfate in alkaline solution, is converted to the marrianolic acid methyl ether by oxidative fission with hypoidite[6] in methanol solution. Esterification[6] is effected with ethereal diazomethane, and the product is purified by chromatography on silica gel.

2. The reaction flask is attached to a vacuum manifold and equipped with a tight fitting rubber stopper of the type used with serum bottles. Additions are made with a hypodermic syringe.

3. The dried ether solution contains 2.80 mmoles of triphenylmethane (m.p. 90.5–92.5°) and 0.190 g. of starting material (m.p. 70.5–74°), which are separated by chromatography.

4. The gas is swept through a trap containing 0.5 *N* sodium hydroxide to recover carbon-C^{14} dioxide. Decarboxylation commences at 168°; the recovery of barium carbonate-C^{14} is 0.517 g. (87%), or 44.7% radiochemical recovery.

5. The residue is chiefly C_1^{14}-marrianolic anhydride methyl ether.

6. The mixed melting point and infrared spectrum agree with those of the starting material.

7. The pure compound melts at 153–154°.

8. In contrast to this homogeneous system,[7] the usual heterogeneous reaction conditions for the acyloin condensation (finely dispersed sodium in boiling xylene) do not give the product in recognizable amounts. Reinvestigation indicates that the configuration of the 17-hydroxyl is β.

9. The desulfuration of mercaptals is a convenient and mild method of preparative reduction of steroid ketones.[8]

10. Recrystallization raises the melting point to 137–138°.

11. The pure compound melts at 120–120.5°. 17β-Estradiol-16-C^{14} 3-methyl ether 17-acetate is formed by storing overnight a mixture of 40 mg. of (d), 1 ml. of pyridine and 0.5 ml. of acetic anhydride, and then pouring the solution into 25 ml. of ice-water. The ester melts at 101–102.4°, after several recrystallizations from alcohol.

12. The diacetate and dipropionate melt at 125–127° and 106–107°, respectively. The purification of 17β-estradiol by chromatography and countercurrent distribution is described by Beer.[11]

C. Other Preparations

17β-Estradiol-16-C^{14} has been prepared by Heard.[9] The D ring was reconstituted[5,10] by employing diazomethane-C^{14} in the Arndt-Eistert reaction to extend the side chain of the half ester of marrianolic acid methyl ether; then the dimethyl ester of homomarrianolic methyl ether was converted to the lead salt, which cyclized at 270° in 65% yield (15% overall yield of estrone on a semimicro scale). The reduction of estrone-16-C^{14} was accomplished with lithium aluminum hydride.[12]

[1] *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 607.

[2] J. C. Sheehan, R. C. Coderre, L. A. Cohen and R. C. O'Neill, J. Am. Chem. Soc., *74*, 6155 (1952).

[3] M. N. Huffman and M. H. Lott, J. Am. Chem. Soc., *71*, 719 (1949).

[4] H. Adkins and H. R. Billica, J. Am. Chem. Soc., *70*, 695 (1948).

[5] G. Anner and K. Miescher, Helv. Chim. Acta, *31*, 2173 (1948).

[6] J. Heer and K. Miescher, Helv. Chim. Acta, *28*, 156 (1945).

[7] M. S. Kharasch, E. Sternfeld and F. R. Mayo, J. Org. Chem., *5*, 362 (1940).

[8] H. Hauptmann, J. Am. Chem. Soc., *69*, 562 (1947).

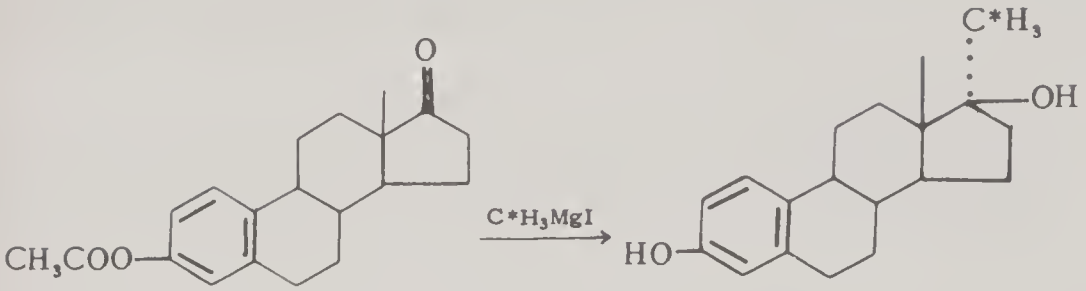
[9] M. Levitz, J. Am. Chem. Soc., *75*, 5352 (1953); R. D. H. Heard, *et al.*, Intern. Congr. Biochem., Abstrs. Communs., 1st Congr., Cambridge, Engl., *1949*, 428; Chem. Abstracts, *47*, 2192 (1953); R. Heard, J. Saffran and L. Thompson, Nucleonics, 7 (4), 52 (1950).

[10] F. Litvan and R. Robinson, J. Chem. Soc., *1938*, 1997.

[11] C. T. Beer and T. F. Gallagher, J. Biol. Chem., *214*, 331, 351 (1955).

[12] G. Papineau-Couture, E. M. Richardson and G. A. Grant, Can. J. Research, *27B*, 902 (1949).

17α-METHYL-C^{14}-17β-ESTRADIOL



B. C. Blocklage, H. J. Nicholas, E. A. Doisy, Jr., W. H. Elliott, S. A. Thayer and E. A. Doisy, J. Biol. Chem., *202*, 27 (1953); Federation Proc., *9*, 209 (1950).

A. Procedure (Note 1)

To a stirred Grignard solution prepared from 0.092 g. of magnesium powder, 3 ml. of absolute ether and 4 mmoles of methyl-C^{14} iodide is added slowly 0.2499 g. (0.8 mmole) of estrone acetate in about 8 ml. of anhydrous toluene (Note 2). The white gel that forms is stirred under nitrogen at room temperature for 30 minutes, then is refluxed for 3 hours and stored overnight. The complex is hydrolyzed with 2% ammonium chloride solution containing a little hydrochloric acid. The ether extract is evaporated, and the oily residue is treated twice with Girard's reagent T to remove ketonic material (Note 3). The nonketonic residue (0.222 g.) is chromatographed on a magnesium trisilicate-Celite (1 : 1) column and eluted with petroleum ether containing absolute ethanol (0.5% by vol.). The product is recrystallized successively from benzene-petroleum ether (b.p. 110–115°), methanol-petroleum ether (b.p. 30–60°), aqueous methanol, and aqueous acetone; yield 40%, m.p. 195–196° (Note 4).

B. Notes

1. Although 17α-methyl-17β-estradiol 3-methyl ether may be prepared in 80% yield from estrone methyl ether by the Grignard reaction, the yield of 17-methylestradiol is poor because of unsatisfactory methods of hydrolysis. The readily hydrolyzed estrone acetate is employed with an excess of Grignard reagent, some of which is consumed in hydrolysis of the ester bond.[1]

2. The ether-solubility of the starting material (m.p. 125–126°) is poor.

3. (Carboxymethyl)trimethylammonium chloride hydrazide[2] forms water-soluble compounds with substances containing the carbonyl group.[3]

4. The biological activity of this product appears to be consistent with the 17α-methyl-17β-ol configuration. The monobenzoate, prepared with

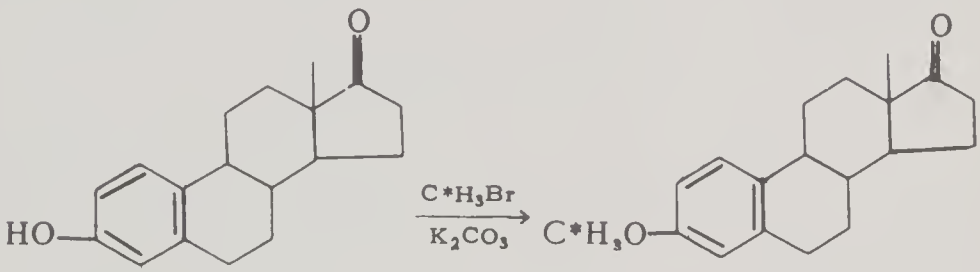
benzoyl chloride and pyridine, melts at 213–214.5°. 17-Methylestradiol monoacetate, prepared with acetic anhydride and pyridine, melts at 148–149.5°.

[1]S. B. Binkley, D. W. MacCorquodale, S. A. Thayer and E. A. Doisy, J. Biol. Chem., *130*, 219 (1939).

[2]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 85.

[3]A. Girard and G. Sandulesco, Helv. Chim. Acta, *19*, 1095 (1936).

ESTRONE METHYL-C^{14} ETHER



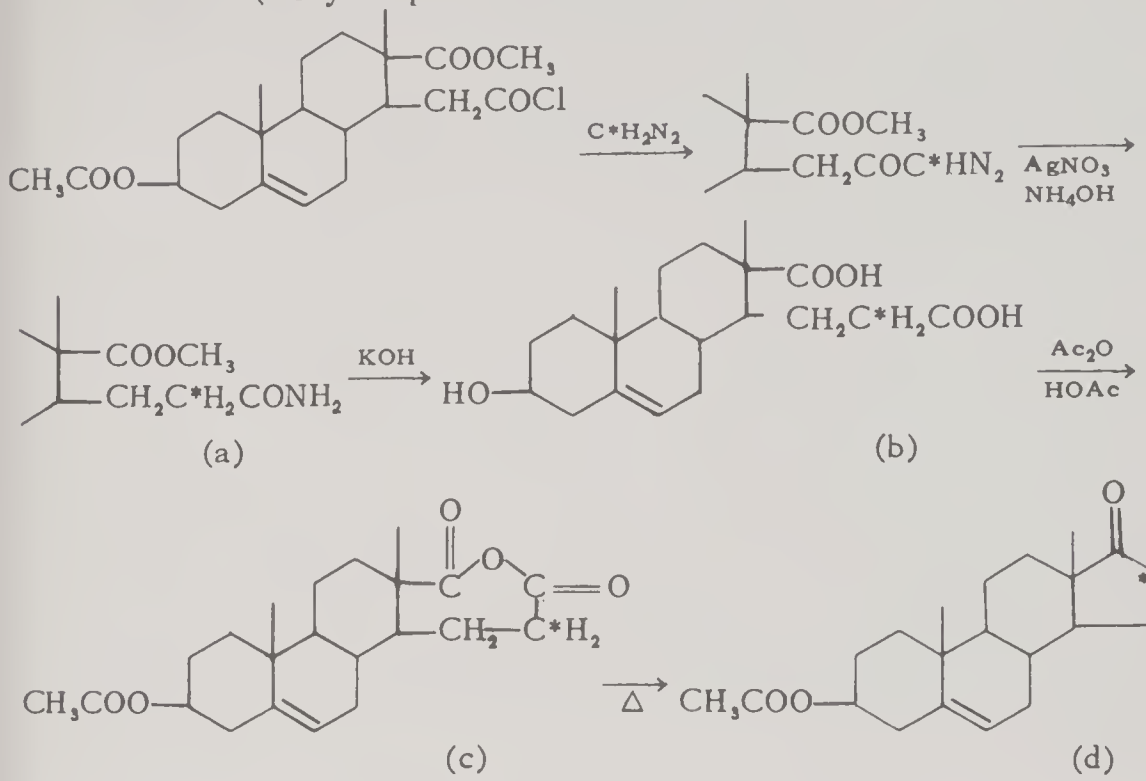
A. Murray III, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, unpublished work.

Procedure

The procedure is an adaptation of the method of Claisen[1] for preparing aryl ethers. The apparatus consists of a round-bottomed flask equipped with a glass-enclosed magnetic stirring bar, a sealed-on reflux condenser, and attachment to a vacuum manifold. To a mixture, frozen in liquid nitrogen, of 0.323 g. (1.19 mmoles) of estrone, 0.157 g. of pulverized anhydrous potassium carbonate and 20–25 ml. of dry acetone is added 0 0363 g. (1.13 mmoles) of methyl-C^{14} bromide by vacuum distillation. The system is partially filled with dry air, and the reactor is sealed off from the manifold by means of a torch at an appropriate constriction. The mixture is stirred overnight at room temperature and then for 8 hours at 80° under reflux. The mixture is distilled to dryness with recovery of 11.9% unreacted methyl bromide. The residue is stirred with 130 ml. of 5% warm potassium hydroxide solution, then collected (cool) on a filter, washed with alkali and water, and dried. The crude product is dissolved in acetone, and the filtered solution is evaporated and dried in a vacuum desiccator; yield 0.2914 g. (85.2%). The product is purified by recrystallization from 16 ml. of absolute alcohol at 0°; yield 0.257 g. (75%), m.p. 165–168°.

[1]L. Claisen, Ann., *418*, 78 (1918).

3β-HYDROXY-5-ANDROSTEN-17-ONE-16-C^{13} ACETATE
(Dehydroepiandrosterone-16-C^{13} Acetate)



E. B. Hershberg, E. Schwenk and E. Stahl, Arch. Biochem., *19*, 300 (1948).

A. Procedure

(a)*7-Acetoxy-2-carbomethoxy-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro-2,4b-dimethyl-1-phenanthrenepropionamide-α-C^{13}, (3β-Acetoxy-16-homo-5-etiobilienamide-16-C^{13} 17-Methyl Ester)* (Note 1). To a cold solution of 15 mmoles of diazomethane-C^{13} in 65 ml. of anhydrous ether is added a solution of 2.07 g. (5 mmoles) of 7-acetoxy-2-carbomethoxy-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro-2,4b-dimethyl-1-phenanthreneacetyl chloride, (3β-acetoxy-5-etiobilien-16-oyl chloride 17-methyl ester), (Note 2) in 70 ml. of ether. The mixture is stored overnight at −5°, then evaporated at the water pump. To a solution of the residual diazoketone in 170 ml. of 95% ethanol at 50–55° is added a solution of 0.9 g. of silver nitrate in 17 ml. of concentrated aqueous ammonia, and the dark mixture is refluxed for 1.5 hours, then concentrated to small volume. The solution is diluted with water to the point of incipient turbidity at the boiling point, then treated with 1 ml. of saturated sodium chloride solution and 1–2 g. of charcoal, and filtered by gravity. The hot filtrate is saturated with water and slowly cooled to crystallize the product; yield 1.7 g. (83%), m.p. 161–163.5° (Note 3).

(b) *2-Carboxy-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro-7-hydroxy-2,4b-dimethyl-1-phenanthrenepropionic-α-C^{13} Acid, (3β-Hydroxy-16-homo-5-*

etiobilienic-16-C^{13} Acid). A mixture of 0.62 g. of the amide, 10 ml. of methanol and 1 g. of potassium hydroxide is refluxed for 1 hour, then diluted with 10 ml. of water, and the heating is continued overnight. The aqueous solution is poured into cold dilute acid, and the precipitated product is collected and recrystallized from dilute methanol; yield 0.44 g. (80%), m.p. 248–249.5°.

(c) *3β-Acetoxy-2-carboxy-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro-2,4b-dimethyl-1-phenanthrenepropionic-α-C^{13} Acid Anhydride, (3β-Acetoxy-16-homo-5-etiobilienic-16-C^{13} Anhydride*) (Note 4). A mixture of 0.500 g. of the acid, 30 ml. of acetic acid and 2 ml. of acetic anhydride is refluxed for 10 hours. Most of the solvent is distilled at atmospheric pressure, the last traces being removed under vacuum.

(d) *3β-Hydroxy-5-androsten-17-one-16-C^{13} Acetate* (Note 5). The residue of crude anhydride is pyrolyzed by heating for 15 minutes at 250–265° at about 15 mm. in a flask with a sealed-on receiver. The product then is distilled at 0.5–2 μ and collected as the semicarbazone; yield 0.27 g., 47% (Note 6). Hydrolysis of 0.307 g. of the semicarbazone in dioxane with sulfuric acid gives 0.200 g. of product, m.p. 168–169.5° (Note 7).

B. Notes

1. The method is based upon the Arndt-Eistert procedure[1] for lengthening a carboxylic acid chain. The reaction involves a rearrangement[2] in which the incoming carbon occupies the alpha position of the new acid, and the carbonyl carbon of the starting acid becomes the carbonyl carbon of the final acid (see phenylacetic-1-C^{13} acid).

2. Directions are given by Hershberg for preparing the starting material by opening the steroid D ring of the structure of the final compound by oxidative cleavage, followed by partial esterification of the resulting dicarboxylic acid, and halogenation.

3. In large-scale experiments the yield varies from 60–77%. The isotopic concentration agrees with theory.

4. The procedure is a modification of the method of Kuwada.[3] The average yield of dehydroepiandrosterone acetate is increased from 25% to 47% by preparing the anhydride in diluted acetic anhydride.

5. The cyclization procedure is adapted from the method of Blanc.[4]

6. Some high-boiling resin remains in the pyrolysis flask.

7. The pure compound melts at 170.2–170.8°. The atom per cent excess C^{13} is 0.104 (calculated, 0.095).

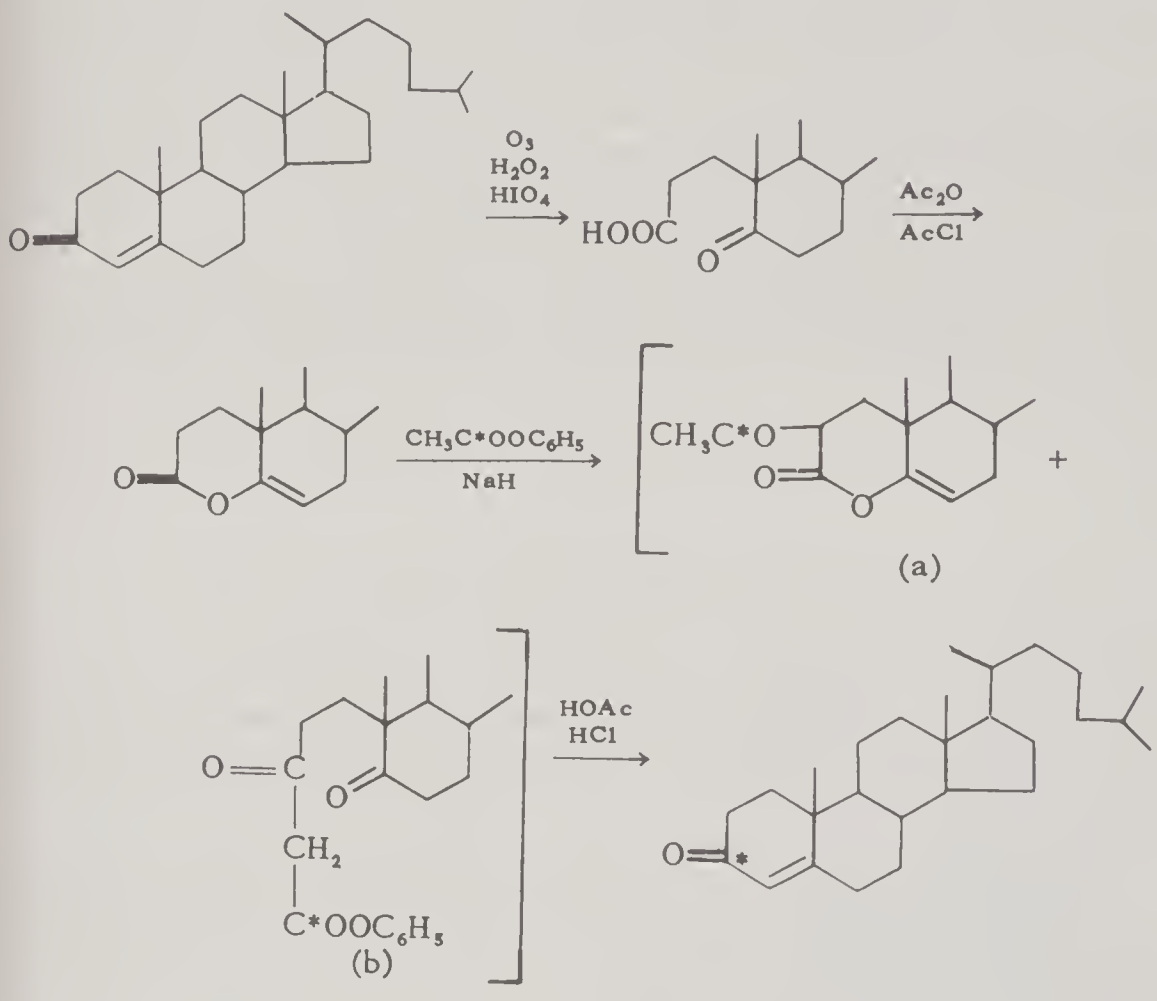
[1] *Organic Reactions*, Vol. I, Wiley, New York, 1942, p. 38.

[2] C. Huggett, R. T. Arnold and T. I. Taylor, J. Am. Chem. Soc., 64, 3043 (1942).

[3]S. Kuwada and K. Nakamura, J. Pharm. Soc. Japan, *58*, 235, 257, 835 (1938); Chem. Abstracts, *33*, 2531 (1939).

[4]H. G. Blanc, Compt. rend., *144*, 1356 (1907).

4-CHOLESTEN-3-ONE-3-C^{14}



R. B. Turner, J. Am. Chem. Soc., *72*, 579 (1950); *69*, 726 (1947).

A. Procedure

A flask containing 0.133 g. (5.54 mmoles) of sodium hydride, 0.715 g. (1.85 mmoles) of 2,3,3a,4,5,5a,6,9,9a,9b-decahydro-7-hydroxy-3a,6-dimethyl-3-(1,5-dimethylhexyl)-1*H*-benz[*e*]indene-6-propionic acid δ-lactone (Note 1), 3.00 ml. of anhydrous benzene and 0.500 g. (3.67 mmoles) of phenyl acetate-1-C^{14} (Note 2) is attached to a buret system filled with dry nitrogen. The mixture is stirred (magnetically) at room temperature

until hydrogen evolution ceases (42 hours, 89%). The mixture is acidified with dilute hydrochloric acid, and the condensation product is extracted into ether, which then is washed with water and saturated sodium chloride, filtered through anhydrous sodium sulfate, and concentrated under reduced pressure. The volatile by-products (Note 3) are distilled off at 100° (0.01 mm.), and the residue is refluxed for 27 hours in a slow stream of nitrogen with a mixture of 25 ml. of acetic acid and 3 ml. of concentrated hydrochloric acid (Note 4). The mixture is diluted with water and extracted into ether, which then is washed with water, dilute sodium hydroxide and saturated sodium chloride solution, then filtered through anhydrous sodium sulfate and evaporated to dryness. The residual oil (0.638 g.) is purified by chromatography on 12.0 g. of alumina; yield 0.362 g. (51%), m.p. 78–80° (Note 5). Recrystallization from acetone gives a pure product melting at 80–80.6°; $[\alpha]_D^{26}$ + 86° (*c* 1.01, $CHCl_3$). The molar specific activity is 90% of that of the starting material (Note 5).

B. Notes

1. Detailed directions are given by Turner for the preparation of this enollactone starting material. Cholestenone is treated with ozone, and the ozonide is converted[1] to dodecahydro-3a,6-dimethyl-3-(1,5-dimethylhexyl)-7-oxo-1*H*-benz[*e*]indene-6-propionic acid (80–85% yield) by oxidation with hydrogen peroxide and treatment of neutral fractions with periodic acid. The enol-lactone then is prepared by heating the keto acid with acetyl chloride and acetic anhydride; yield 92%, m.p. 94–94.5°.

2. The use of a very large excess of phenyl acetate does not appreciably increase the yield, but the figure drops to about 36% when equimolar quantities are employed. Havinga[2] has reported a yield of 3.25 g. of 4-cholesten-3-one-3-C^{14} based on 4.036 g. of phenyl acetate-1-C^{14} and 5.77 g. of dodecahydro-3a,6-dimethyl-3-(1,5-dimethylhexyl)-7-oxo-1*H*-benz[*e*]indene-6-propionic acid.

3. Phenol, phenyl acetoacetate.

4. The evolved carbon dioxide is collected in an alkali trap and precipitated as the barium salt (1.21 mmoles). This hydrolysis, decarboxylation and cyclization also may be accomplished by the action of dilute alkali.

5. While both intermediates (a) and (b) lead to cholestenone with the methyl carbon of phenyl acetate occupying position 4, only intermediate (a) permits the carbonyl group to phenyl acetate to become a part of the reformed ring. Thus, 90% of the product must be formed through intermediate (a).

C. Other Preparations

4-Cholesten-3-one-4-C^{14} is prepared by the same procedures from phenyl acetate-2-C^{14}. There is no dilution in molar specific activity.

Turner[3] has prepared 4-cholesten-3-one-3-C^{14} in 15% yield (47% cor.) by condensing the methyl ester of keto acid (Note 1) with 100% excess methyl bromoacetate-1-C^{14} by the Reformatsky reaction,[4] followed by cyclization, hydrolysis and decarboxylation. Also see 3-oxo-4-etiocholenic-3-C^{14} acid.

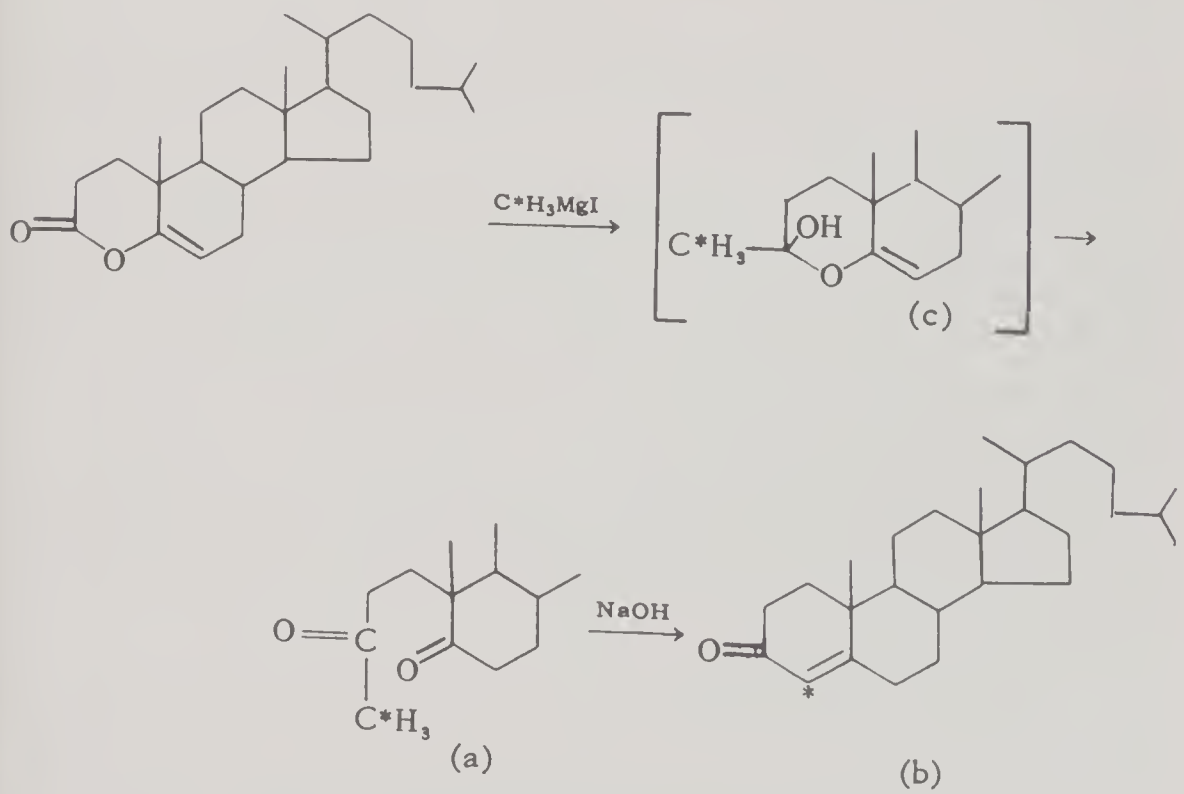
[1]C. C. Bolt, Rec. trav. chim., *57*, 905 (1938); M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert, P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 238.

[2]E. Havinga and P. P. L. Bots, Rec. trav. chim., *73*, 393 (1954).

[3]R. B. Turner, J. Am. Chem. Soc., *72*, 579 (1950).

[4]R. D. H. Heard, *et al.*, Intern. Congr. Biochem., Abstrs., Communs., 1st. Congr., Cambridge, Engl., *1949*, 428; Chem. Abstracts, *47*, 2192 (1953).

4-CHOLESTEN-3-ONE-4-C^{14}



G. I. Fujimoto, J. Am. Chem. Soc., 73, 1856 (1951).

A. Procedure

(a) *3-(1,Dimethylhexyl)decahydro-3a,6-dimethyl-6-(3-oxobutyl-4-C^{14})-1H-benz[e]indene-7(2H)-one, (4,5-Seco-3,5-cholestanedione-4-C^{14}).* To a stirred solution of 0.386 g. (1 mmole) of the enol-lactone (Note 1) in 3 ml. of ether at 0°, under an atmosphere of nitrogen, is slowly added dropwise the Grignard reagent prepared from 0.142 g. (1 mmole) of methyl-C^{14} iodide. The mixture is left in the cold for 20 hours, then decomposed with 3 ml. of saturated ammonium chloride solution. The ether extract is washed with dilute sodium carbonate and water, then evaporated to dryness. The crude product is recrystallized repeatedly from acetone. The yield of colorless plates is 45–65%, m.p. 174–178° (Note 2); see progesterone-4-C^{14}.

(b) *4-Cholesten-3-one-4-C^{14}.* To the crude mixture from the Grignard reaction (above) is added 30 ml. of methanol, 5 ml. of water and 1 g. of sodium hydroxide; then the mixture is set aside for 24 hours with occasional stirring. The crude product is obtained by evaporation of the methanol, followed by washing with alkali (Note 3). Purification by chromatography on alumina yields two fractions: m.p. 80–82°, from petroleum ether-benzene (9 : 1); m.p. 86–88°, from petroleum ether-benzene (4:1) (Note 4). The yield is 52–60% based on methyl iodide (Note 5).

B. Notes

1. Directions are given by Turner[1] for preparing this starting material; see 4-cholesten-3-one-3-C^{14}.

2. Heard and Ziegler,[2] by a quite similar procedure, at this point isolated a compound (52% yield) melting at 160–175° (unchanged by repeated recrystallization), which they characterized as the intermediate hemiketal (c), 3-hydroxy-3-methyl[-C^{14}-]-4-oxa-5-cholestene. It is converted to cholestenone by treatment with potassium hydroxide, hydrochloric acid or piperidine, or by distillation at 220–230° (0.1 mm.). A sample distilled at 170–180° (0.1 mm.) gave a product which could not be crystallized, but could be converted to cholestenone by treatment with alkali, still gave a positive Zimmermann reaction, no longer gave a coloration with tetranitromethane, and possessed a value $[\alpha]_D^{19}$ + 60° ($CHCl_3$) as against $[\alpha]_D^{23}$ + 10° ($CHCl_3$). This compound gave a bis(2,4-dinitrophenylhydrazone) (m.p. 243–245°) that was identical to that from (c). These investigators have characterized this second intermediate compound as (a).

3. Heard and Ziegler[2] isolate the product by evaporation of the methanol, dilution with water and extraction into ether solution, which is then washed, dried with sodium sulfate and evaporated. The product is crys-

tallized from ether-methanol in high yield, m.p. 80–81°. Stokes[3] has purified the product by chromatography on alumina, m.p. 82–83°.

4. The melting point of either fraction is not depressed upon mixed melting with an authentic sample, and the infrared spectra of all three samples are identical.

5. A yield of 55% has been reported with this procedure by Dauben, according to Fujimoto.

C. Other Preparations

Heard and Ziegler[2] have prepared 4-cholesten-3-one-4-C^{14} (m.p. 76–78°) in 45% yield on the 3.74-mmole scale by essentially the same reaction.

Turner[1] has prepared 4-cholesten-3-one-4-C^{14} in 51% yield (m.p. 78–80°) on the 3.67-mmole scale by reacting the enol-lactone with phenyl acetate-2-C^{14} in the manner described under 4-cholesten-3-one-3-C^{14}.

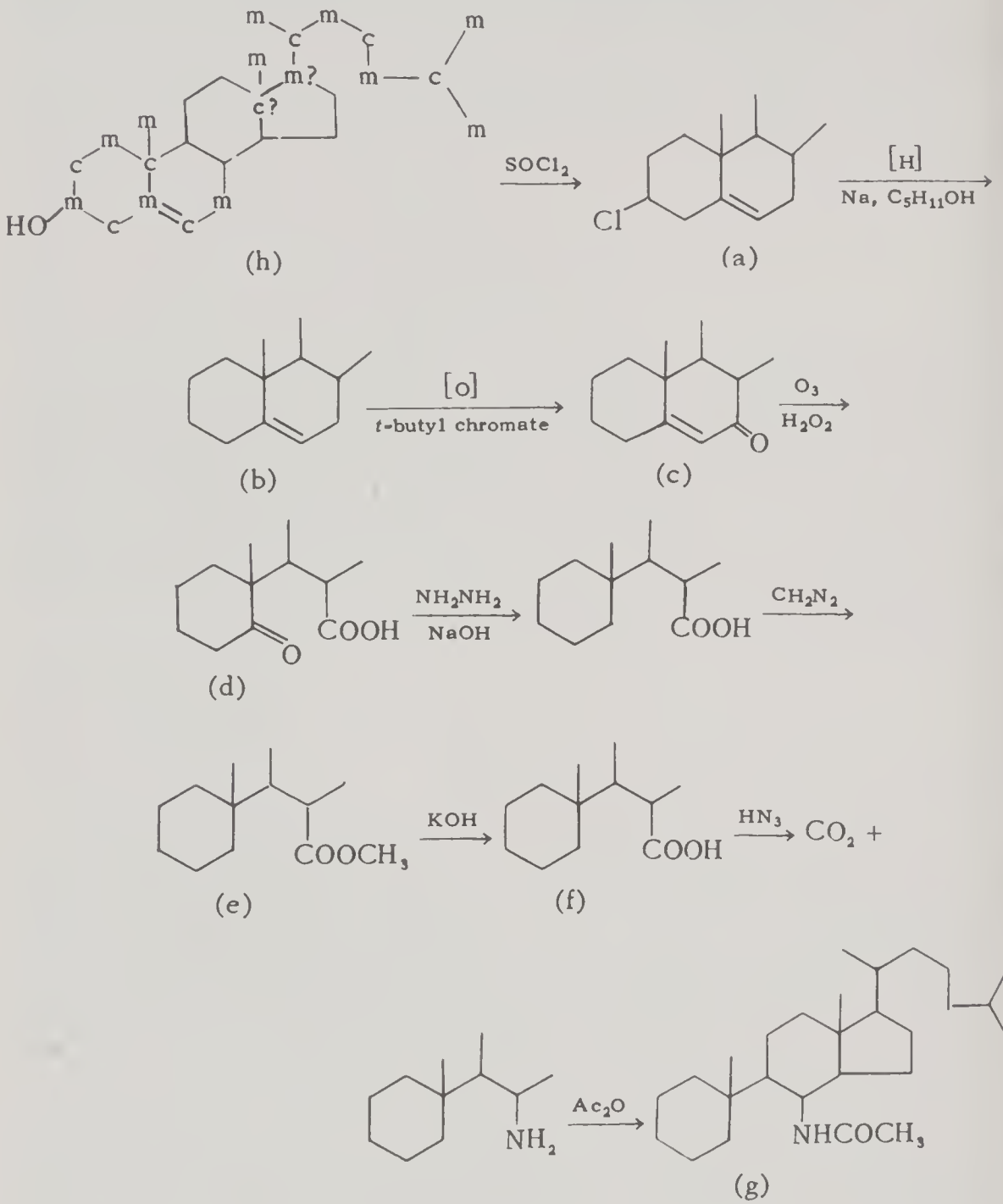
4-Cholesten-3-one-3-(and/or)-4-C^{14} have been prepared[4] by the Reformatsky reaction of 3,5-seco-4-nor-5-oxocholestan-3-oic acid with labeled ethyl bromoacetate (see 3-oxo-4-etiocholenic-3-C^{14} acid).

Comparative yields[2] for the three methods for labeling cholestenone in the A ring (based on the ring A keto acid) are: Grignard reaction 70%, phenyl acetate condensation 45%, and Reformatsky reaction 30%; based on isotopic carbon the respective yields are: 45–70% (based on methyl iodide), 22%, and 10% (both based on sodium acetate).

The preparation of 4-cholesten-3-one-4-C^{14}, by employing the inverse order of Grignard reagent addition and without isolating compound (a), has been described by Hunziker.[5] Purification was accomplished by chromatography on aluminum oxide, the eluant being petroleum ether; yield 63% based on the enol lactone at the 10-mmole level. For preparation of the "inactive" aluminum oxide, see vitamin-D_3-4-C^{14}, Note 10.

[1]R. B. Turner, J. Am. Chem. Soc., *72*, 579 (1950).
[2]R. D. H. Heard and P. Ziegler, J. Am. Chem. Soc., *73*, 4036 (1951).
[3]W. M. Stokes, W. A. Fish and F. C. Hickey, J. Biol. Chem., *213*, 325 (1955).
[4]R. D. H. Heard *et al.*, Intern. Congr. Biochem., Abstrs. Communs., Ist Congr., Cambridge, Engl., *1949*, 428; Chem. Abstracts, *47*, 2192 (1953).
[5]F. Hunziker, Helv. Chim. Acta, *38*, 917 (1955).

{C^{14}-*N*-[1-(1,5-DIMETHYLHEXYL)HEXAHYDRO-7a-METHYL-5-(1-METHYLCYCLOHEXYL)-4-INDANYL]}ACETAMIDE



W. G. Dauben and K. H. Takemura, J. Am. Chem. Soc., 75, 6302 (1953).

A. Procedure (Note 1)

(a) *C^{14}-3β-Chloro-5-cholestene, (C^{14}-Cholesteryl Chloride)* (Note 2). A mixture of 3.600 g. of C^{14}-cholesterol and 10 ml. of purified thionyl

chloride is stored at room temperature for 2 hours and evaporated at 35–40° under reduced pressure, the excess reagent being completely removed by the evaporation of 10 ml. of added dry benzene. The residue is crystallized from 12 ml. of hot, dry acetone by cooling at 0° and finally at −70°; yield 3.642 g. (94.5%), m.p. 93.0–95.5° (Note 3).

(b) *C^{14}-5-Cholestene* (Note 4). To a boiling solution of 3.64 g. of C^{14}-cholesteryl chloride in 80 ml. of isopentyl alcohol is added 6.3 g. of sodium. The resulting solution is cooled, diluted with water and extracted with ether. The extract is washed with water, dilute hydrochloric acid, water, sodium bicarbonate, water and saturated sodium chloride solution, then dried over sodium sulfate and concentrated at 100° under vacuum. The residue is recrystallized from methanol; yield 3.21 g. (96.5%), m.p. 89.5–91.2° (Note 5).

(c) *C^{14}-7-Oxo-5-cholestene.* The 5-cholestene is oxidized with *t*-butyl chromate (Note 6) according to the procedure of Heusler and Wettstein,[1] which follows for the preparation of 3β,17β-diacetoxy-7-oxo-5-androstene. The crude product is purified by chromatography on alumina. Elution with hexane gives 1.13 g. of starting material, and elution with ether-hexane (10:90) gives the product; yield 0.879 g. (41% based on unrecovered starting material), m.p. 125–129°.

To a stirred mixture of 89.0 g. of 3β,17β-diacetoxy-5-androstene in 500 ml. of dry carbon tetrachloride at 80° is added dropwise, during 30 minutes, a mixture containing 720 ml. of the *t*-butyl chromate solution, 225 ml. of glacial acetic acid and 90 ml. of acetic anhydride. The mixture is stirred for 10 hours at 80°, then cooled with an ice-bath and treated dropwise with a solution of 150 g. of oxalic acid in 1.5 l. of water during 45 minutes. The mixture is stirred for 15 minutes, then treated with 105 g. of solid oxalic acid and stirred for 2 hours as the temperature slowly rises to that of the room. The aqueous layer is extracted with carbon tetrachloride, and the combined extract is washed with water, sodium bicarbonate and water, dried and evaporated. The crude product (89.0 g.) is dissolved in 9.0 l. of boiling ether, and the clear solution is concentrated and cooled, giving 55.8 g. of the oxo compound (60.4% yield).

(d) *C^{14}-1-(1,5-Dimethylhexyl)hexahydro-7a-methyl-5-(1-methyl-2-oxocyclohexyl)-4-indancarboxylic Acid,* (*C^{14}-5-Oxo-5,7-seco-6-nor-7-cholestanecarboxylic Acid*). Through an ice-cold solution of 0.879 g. of the unsaturated ketone in 100 ml. of ethyl acetate-acetic acid (1:1 by vol.) is passed ozone for 20 minutes at the rate of 0.5 mmole/min. The mixture is diluted with 10 ml. of 3% hydrogen peroxide and stored overnight at room temperature, then heated on a steam-bath for 30 minutes and evaporated at 100° under reduced pressure. The residue is dissolved in ether and extracted with dilute aqueous-methanolic potassium hydroxide. Acidification gives the product, which is collected, washed with water,

dried and recrystallized from ether-hexane; yield 0.403 g. (43.5%), m.p. 180–186° (Note 7).

(e) *Methyl* C^{14}*-(1,5-Dimethylhexyl)hexahydro-7a-methyl-5-(1-methylcyclohexyl)-4-indancarboxylate, (Methyl* C^{14}*-5,7-Seco-6-nor-7-cholestanecarboxylate).* A mixture of 0.655 g. of the keto acid, 1.65 g. of sodium hydroxide, 1.50 ml. of 85% hydrazine hydrate and 15 ml. of diethylene glycol is refluxed for 1 hour (bath 135°); then the vapors are permitted to escape until an internal temperature of 195° (bath 215°) is reached. The mixture is heated under reflux for 5.5 hours, then cooled, diluted with 150 ml. of water, acidified with concentrated hydrochloric acid and extracted with ether. The extract is evaporated, and the dark yellow, oily residue (0.652 g.) is treated with excess diazomethane. The resulting mixture is chromatographed in hexane solution on 15 g. of alumina, and the product is eluted with hexane; yield 0.205 g. (31.4%), m.p. 75–78° (Note 8).

(f) C^{14}*-(1,5-Dimethylhexyl)hexahydro-7a-methyl-5-(1-methylcyclohexyl)-4-indancarboxylic Acid, (*C^{14}*-5,7-Seco-6-nor-7-cholestanecarboxylic Acid).* The ester is hydrolyzed by refluxing under nitrogen for 4 hours in ethylene glycol containing 5% potassium hydroxide. The product is recrystallized from methanol, m.p. 129.0–130.2°.

(g) { C^{14}-N-[*1-(1,5-Dimethylhexyl)hexahydro-7a-methyl-5-(1-methylcyclohexyl)-4-indanyl*]} *acetamide,* [*8-Acetylamino(*C^{14}*-5,8-seco-6,7-bisnorcholestane)*]. To a stirred, ice-cooled mixture of 0.192 g. of the C^{14}-acid, 4 ml. of chloroform and 1.5 ml. of concentrated sulfuric acid is added 0.065 g. of sodium azide in portions (Note 9). The mixture is stirred for 1 hour at 0° and at room temperature for 17 hours, then diluted with ice. The chloroform layer is washed with water, dilute sodium bicarbonate, water and saturated sodium chloride, then filtered through alumina and evaporated. The crude C^{14}-(1,5-dimethylhexyl)hexahydro-7a-methyl-5-(1-methylcyclohexyl)-4-indanamine, (C^{14}-8-amino-5,8-seco-6,7-bisnorcholestane), is stored for 18 hours with 5 ml. of acetic anhydride; then the mixture is diluted with water and extracted with chloroform. The product is recrystallized from aqueous ethanol; yield 0.128 g. (65%), m.p. 185–187°. The molar specific activity is the same as that of the starting materials.

B. Notes

1. The preparations are primarily a degradation of C^{14}-cholesterol (to determine C-7) as part of a study of the postulated biosynthetic formation from C^{14}-acetate *via* squalene. Of the 27 carbons in cholesterol, 15 originate from the methyl and 12 from the carboxyl; see formula (h).[3]

2. The procedure is an adaptation of that of Diels.[2]

3. C^{14}-Cholesteryl chloride melts at 95° after two recrystallizations from acetone (84% yield).[3]

4. The procedure is an adaptation of that of Mauthner.[4] Cornforth[3] has described the isotopic preparation; yield 93%, m.p. 93° from ethanol.

5. A stepwise, partial degradation of C^{14}-5-cholestene, designed to study ring A and B, has been described in detail by Cornforth (see C^{14}-2-methylcyclohexanone).

6. The reagent is prepared[1] after the method of Oppenauer.[5] To 468 ml. of stirred, ice-cooled, purified *t*-butyl alcohol is added 185 g. of chromium trioxide in small portions, the temperature being maintained at 25–30°. After 5 minutes of further stirring, the mixture is diluted with 1.3 l. of dry carbon tetrachloride, and the organic layer is separated and dried over sodium sulfate, the salt being washed with 800 ml. of the solvent. The combined filtrate is concentrated to 1.0 l under vacuum at 40–45°, the excess alcohol being removed by azeotropic distillation. The residual *t*-butyl chromate solution may be kept unchanged for several weeks in a refrigerator.

7. Recrystallization from methanol-water raises the melting point to 184–185°. Further purification may be accomplished by sublimation under high vacuum. The molar specific activity is about 97.5% of that of the starting C^{14}-cholesterol. The lost C^{14}-carbon (C-6) has been shown[3] to be derived from an acetate carboxyl atom.

8. Recrystallization from methanol raises the melting point to 80.5–81.0°.

9. The liberated carbon dioxide is swept out in a stream of nitrogen, collected in 3 ml. of carbon dioxide-free 1 *N* sodium hydroxide and precipitated by the addition of 6 ml. of carbon dioxide-free 1 *N* ammonium chloride, followed by 6 ml. of carbon dioxide-free 1 *M* barium chloride; yield 0.091 g. (94%), specific activity 0. Since C-7 is not derived from a carboxyl carbon, one scheme proposed[6] for the direct utilization of squalene in cholesterol biosynthesis is ruled out in favor of that proposed by Woodward[7] and Dauben.[8] Further evidence in favor of this mechanism has been reported by Dauben[8] based upon the oxidative degradation method described by Little.[9,10]

C. Other Preparations

A partial degradation of C^{14}-cholesterol, biosynthesized from acetate-2-C^{14}, has been outlined by Woodward.[7] C^{14}-3β-Cholestanol, prepared by the method of Robinson,[6] was degraded to C^{14}-3β-hydroxy-17-androstanone, (C^{14}-epiandrosterone), which was then oxidized[9,10] to acetic-$C^{14}_{1/2}$ acid under the conditions used for angular methyl group determination. As C-1 represents the average (steroid) C-10,13, and C-2 represents the average C-18,19, isotope was located at C-18,19 and C-10 or 13.

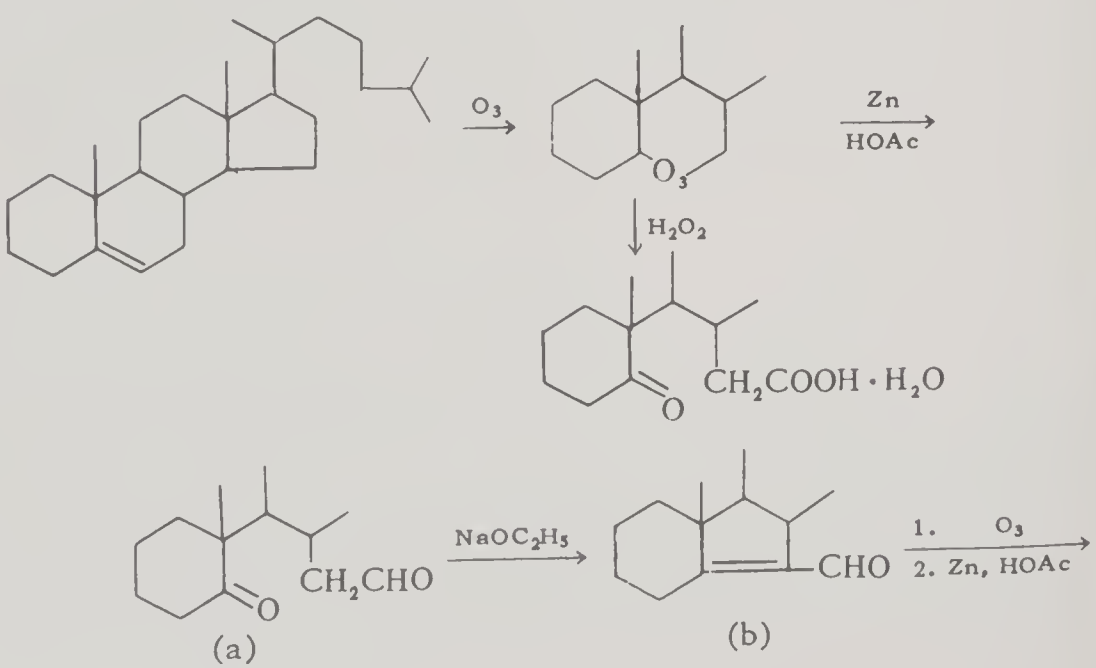
A degradation of C^{14}-cholesterol into nucleus and side chain is described by Little.[10] A stepwise degradation of the side chain is outlined by Wüersch.[11]

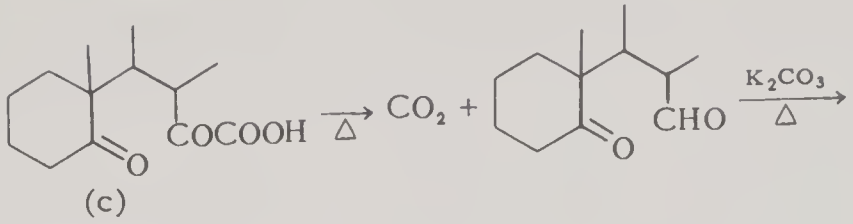
The degradation of C^{14}-cholesterol to C^{14}-allocholanic acid has been outlined by Little.[10] C^{14}-Cholestane was prepared[12] (yield 52–57%) by the sequence: C^{14}-3β-cholestanol, C^{14}-3-cholestanone, and C^{14}-3-cholestanone hydrazone which was reduced[13] by the Wolff-Kishner method. Directions are given for oxidizing the hydrocarbon with chromic acid according to Windaus;[14] yield 10% as the potassium salt. The by-product C^{14}-acetone was isolated as the mercury complex; yield 10–20%.

For the stepwise degradation of C-1,2,3,4,5,6,10 and 19, see C^{14}-2-methylcyclohexanone.

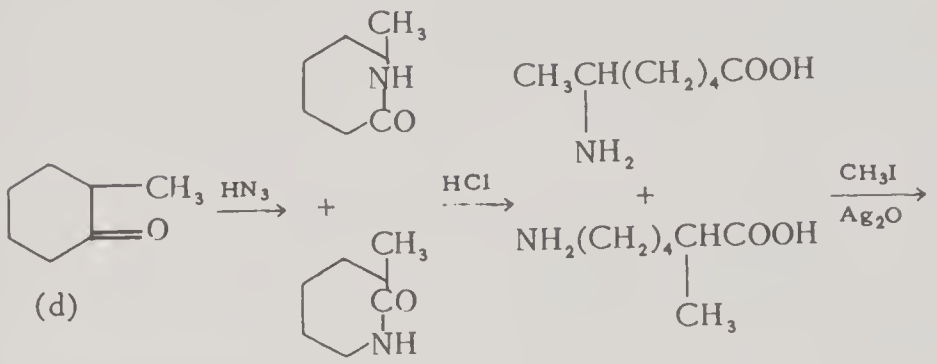
[1]K. Heusler and A. Wettstein, Helv. Chim. Acta, *35*, 284 (1952).
[2]O. Diels and P. Blumberg, Ber., *44*, 2847 (1911).
[3]J. W. Cornforth, G. D. Hunter and G. Popjak, Biochem. J., *54*, 590 597 (1953); Arch. Biochem., *42*, 481 (1953).
[4]J. Mauthner and W. Suida, Monatsch., *15*, 85 (1894).
[5]R. V. Oppenauer and H. Oberrauch, Anales asoc. quim. argentina, *37*, 246 (1949); Chem. Abstracts, *44*, 3871 (1950).
[6]R. Robinson, J. Soc. Chem. Ind., *53*, 1062 (1934).
[7]R. B. Woodward and K. Bloch, J. Am. Chem. Soc., *75*, 2023 (1953).
[8]W. G. Dauben, S. Abraham, S. Hotta, I. L. Chaikoff, H. L. Bradlow and A. H. Soloway, J. Am. Chem. Soc., *75*, 3038 (1953).
[9]R. Kuhn and F. L'Orsa, Angew. Chem., *44*, 847 (1931).
[10]H. N. Little and K. Bloch, J. Biol. Chem., *183*, 33 (1950).
[11]J. Wüersch, R. L. Huang and K. Bloch, J. Biol. Chem., *195*, 439 (1952).
[12]*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 139.
[13]J. D. Dutcher and O. Wintersteiner, J. Am. Chem. Soc., *61*, 1992 (1939).
[14]A. Windaus and K. Neukirchen, Ber., *52*, 1915 (1919).

C^{14}-2-METHYLCYCLOHEXANONE



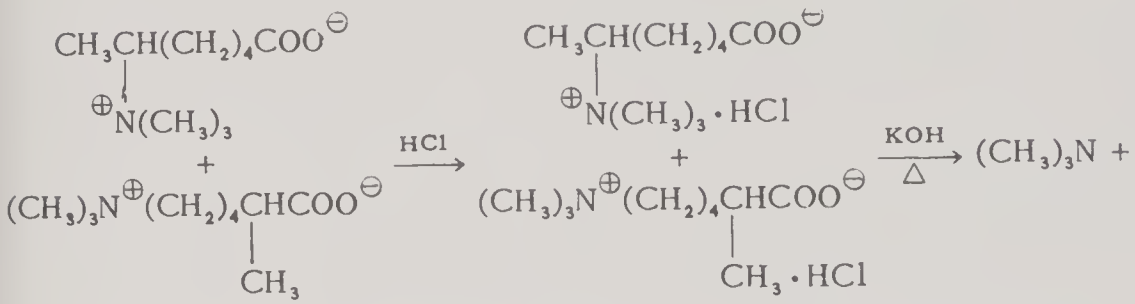


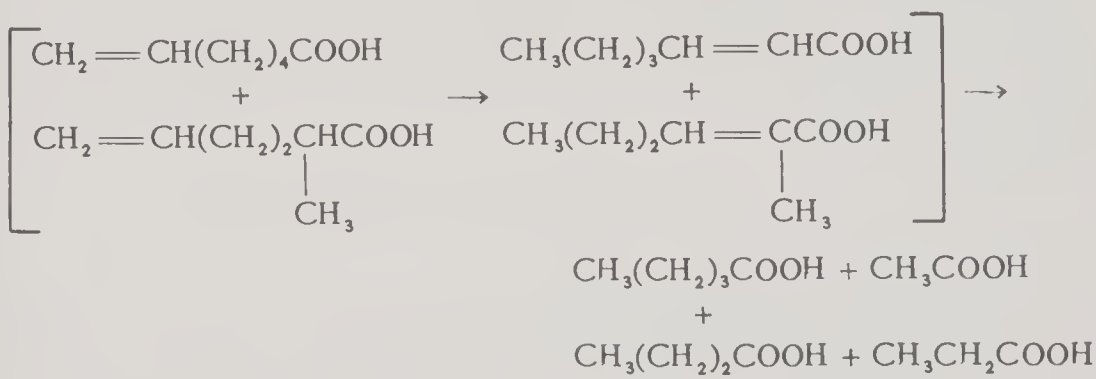
(c)



(d)

(10–15%)





J. W. Cornforth, G. D. Hunter and G. Popják, Biochem. J., *54*, 597 (1953); Arch. Biochem., *42*, 481 (1953).

A. Procedure (Note 1)

(a) *C^{14}-1-(1,5-Dimethylhexyl)hexahydro-5-(1-methyl-2-oxocyclohexyl)-7a-methyl-4-indanacetaldehyde, (C^{14}-5,6-Secocholestan-6-al-5-one).* A solution of 10.0 g. of C^{14}-5-cholestene in 100 ml. of hexane is ozonized with a steady stream of ozone (Note 2) and evaporated under vacuum. This ozonide (Note 3) is reduced by shaking with 10 g. of zinc and 70 ml. of acetic acid for 12 hours at room temperature (Note 4). The inorganic material is separated by filtration and washed with ether; then the

combined filtrate is washed with water, 5% sodium bicarbonate and water and evaporated under vacuum at room temperature; yield 10.1 g. (Note 5).

(b) *C^{14}-1,2,3,3a,4,5,5a,5b,6,7,8,9-Dodecahydro-3-(1,5-dimethylhexyl)-3a,5b-dimethylcyclopenta[a]fluorene-10-carboxaldehyde,* (*C^{14}-6-Formyl-B-norcholest-5(6)-ene*). A solution of 8.8 g. of the secoaldehyde, 90 ml. of absolute alcohol and 10 ml. of 7% ethanolic sodium ethoxide is stored for 24 hours, then poured into dilute acetic acid. The ether layer is washed, dried and concentrated, and the product is isolated by chromatography on alumina with benzene as eluant; yield 8.0 g. (Note 6).

(c) *C^{14}-1-(1,5-Dimethylhexyl)hexahydro-5-(1-methyl-2-oxocyclohexyl)-7a-methyl-4-indanglyoxylic Acid,* (*C^{14}-5,6-Secocholestan-5,7-dion-6-oic Acid*). The noraldehyde (13.5 g.) is ozonized (Note 7) and reduced with zinc and acetic acid as described above, giving 6.6 g. of product, m.p. 57°, and 5.65 g. of a neutral fraction (Note 8).

(d) *C^{14}-2-Methylcyclohexanone.* The diketo acid is heated at 250° under nitrogen for 1.5 hours (Note 9), and the crude product is separated into acidic (chiefly starting material) and neutral (yield 2.3 g.) fractions. The latter fraction is dissolved in light petroleum (b.p. 80–100°) and chromatographed on 69 g. of alumina washed with methyl formate. Elution with light petroleum gives 1.24 g. of C^{14}-1-(1,5-dimethylhexyl)hexahydro-5-(1-methyl-2-oxocyclohexyl)-7a-methyl-4-indancarboxaldehyde, (C^{14}-5,6-seco-B-norcholestan-6-al-5-one), (Note 10).

The mixture of 0.4 g. of the crude neutral product and 1.5 g. of potassium carbonate is heated at 450–500° for 10 minutes; then the combined distillate and residue is neutralized and steam-distilled. The product is isolated as the 2,4-dinitrophenylhydrazone; yield 0.028 g. (10%) (Note 11).

B. Notes

1. The preparations are incidental to a stepwise degradation of biosynthetic C^{14}-cholesterol (prepared from acetate-1- and -2-C^{14}) designed to study ring A and B. The method was suggested by a pyrolysis described by Achtermann[1] and Laucht.[2] Also see Dauben and Takemura, {C^{14}-*N*-[1-(1,5-dimethylhexyl)hexahydro-7a-methyl-5-(1-methylcyclohexyl)-4-indanyl]} acetamide.

2. The elaborate apparatus of Berenstein[3] may be greatly simplified. At the end of the reaction a dilute solution of bromine in acetic acid is no longer rapidly decolorized by mixing with a few drops of the reaction mixture.

3. C^{14}-1-(1,5-Dimethylhexyl)hexahydro-5-(1-methyl-2-oxocyclohexyl)-7a-methyl-4-indanacetic acid, (C^{14}-5,6-secocholestan-5-on-6-oic acid), is obtained by stirring 7.0 g. of the ozonide on a steam-bath with 30 ml. of water and a few ml. of "Perhydrol." The acidic material is isolated

from ether by extraction into 2 *N* sodium hydroxide and acidification; yield of monohydrate 4.9 g., m.p. 61°.

4. Iodine is no longer liberated from a crystal of potassium iodide by a few drops of the reaction mixture.

5. The 2,4-dinitrophenylhydrazone, prepared in 70% yield by treating the viscous oil with ethanolic 2,4-dinitrophenylhydrazine in the presence of hydrochloric acid, melts at 123° from ethanol-*N* hydrochloric acid.

6. $\lambda_{max.}$ 253 mμ; ϵ 6230 in ethanol. The 2,4-dinitrophenylhydrazone melts at 235° from ethyl acetate.

7. Completion of reaction is indicated by disappearance of the yellow color.

8. The neutral fraction is purified by chromatography on 194 g. of alumina washed with methyl formate. Elution with benzene gives 3.5 g. of C^{14}-1-(1,5-dimethylhexyl)hexahydro-5-(1-methyl-2-oxocyclohexyl)-7a-methyl-4-indanglyoxal, (C^{14}-5,6-secocholestan-6-al-5,7-dione), m.p. 142–143° from alcohol-water; the second component is unknown.

9. The acid may be decarboxylated by boiling with aniline, evolving carbon dioxide (cholesterol C-6).

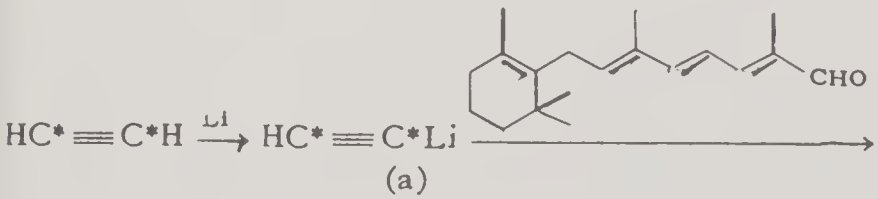
10. 5,6-Seco-B-norcholestan-6-al-5-one melts at 114° from ethanol.

11. A sample of the 2,4-dinitrophenylhydrazone recrystallized from alcohol melted at 127–132°; there was no lowering upon mixed melting with an authentic sample (m.p. 134°). Detailed directions are given for a stepwise degradation of C^{14}-2-methylcyclohexanone by the sequence: C^{14}-2-methyl-7-oxohexamethylenimine (70%, m.p. 90°) and C^{14}-3-methyl-2-oxohexamethylenimine (impure mixture, 10–15% yield), C^{14}-6-amino-heptanoic acid hydrochloride (99%, m.p. 130–132°), C^{14}-(5-carboxy-2-methylpentyl)trimethylammonium chloride (m.p. 146–149° from alcohol) and C^{14}-valeric acid (44%, C-3,2,1,10,19), C^{14}-butyric acid (6%, C-1,2,3,4), C^{14}-propionic acid (6%, C-5,10,19) and C^{14}-acetic acid (43%, C-5,4). The acids were separated by column chromatography (yields based on the crude amino acid) and further degraded stepwise.

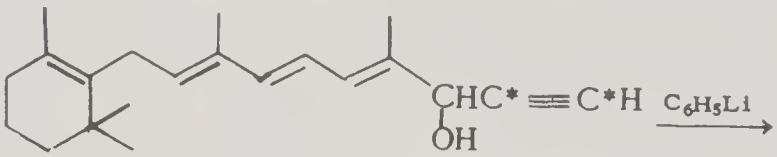
[1]T. Achtermann, Hoppe Seyler's, Z. physiol., *225*, 141 (1934).
[2]F. Laucht, *ibid.*, *237*, 236 (1935).
[3]M. Berenstein, A. Georg and E. Briner, Helv. Chim. Acta, *29*, 258 (1946).

VITAMINS

All-*trans*-β-CAROTENE-15,15′-C_2^{14}
(C_2^{14}-Provitamin A)



(a)



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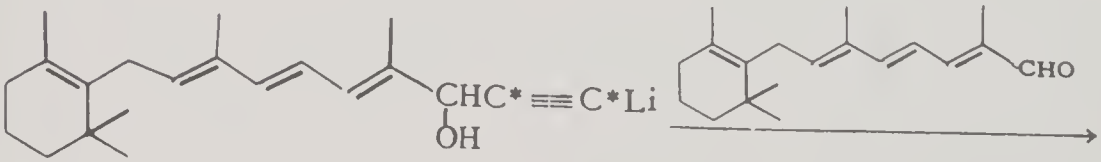

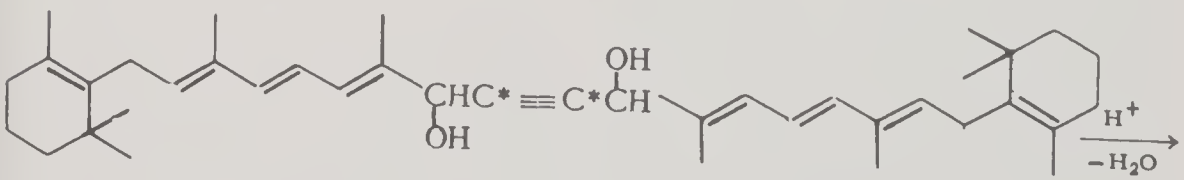

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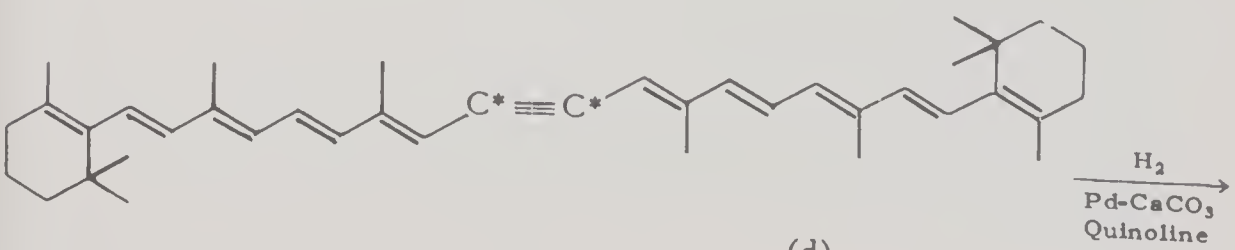
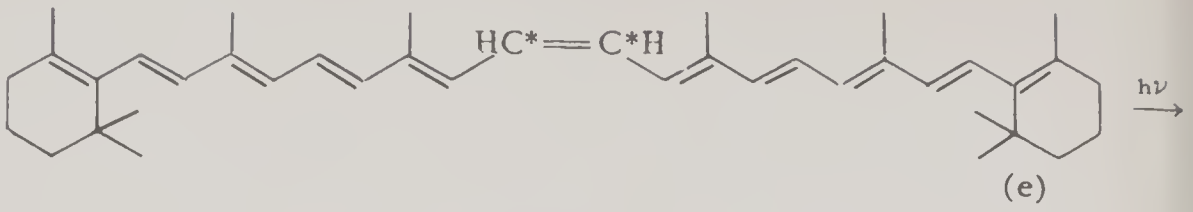
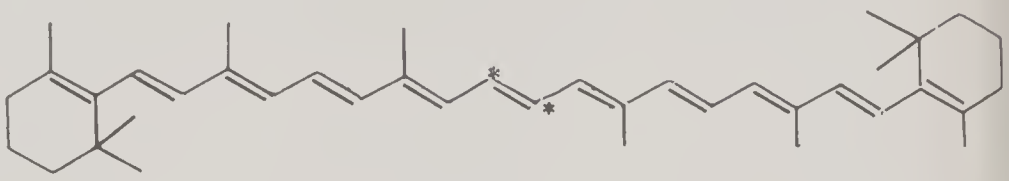


(d)



(e)



(f)

H. H. Inhoffen, U. Schwieter, C. O. Chichester and G. Mackinney, J. Am. Chem. Soc., 77, 1053 (1955).

A. Procedure (Note 1)

(a) *Lithium Acetylide-*C_2^{14}. To a solution of 0.050 g. (7.2 mmoles) of lithium in 125 ml. of liquid ammonia, frozen with liquid nitrogen, is added 0.48 mmole of dry acetylene-C_2^{14} by vacuum transfer. The mixture is thawed and decolorized by the passage of inactive acetylene.

(b) *1-Ethynyl-*C_2^{14}*-2,6-dimethyl-8-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,-4,6-octatrien-1-ol.* To the lithium acetylide-C_2^{14} solution is added dropwise 0.500 g. (1.84 mmoles) of 2,6-dimethyl-8-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6-octatrienal (Note 2) in 30 ml. of absolute ether. The mixture is stirred for 2 hours at $-40°$ in a gentle stream of dry nitrogen under a cold finger reflux condenser (Note 3), then for 1 hour at room temperature as the ammonia slowly evaporates. The mixture is hydrolyzed with 1.0 g. of dry ammonium chloride (Notes 3 and 4), and the residue is dissolved in 50 ml. of ether and washed with two portions of water. The solvent is evaporated under nitrogen, and the crude product is freeze-dried; yield 0.530 g. (Note 5).

(c) *3,7,12,16-Tetramethyl-1,18-bis(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,-4,6,12,14,16-octadecahexaen-9-yne-8,11-diol-9,10-*C_2^{14}. To a solution of the ethynylcarbinol in 25 ml. of absolute ether is added dropwise 0.026 g. (Note 6) of phenyllithium in 50 ml. of ether. The mixture is refluxed for 30 minutes; then 0.500 g. of the C_{19}-aldehyde in 25 ml. of absolute ether is added dropwise, and the turbid solution is refluxed for 1.5 hours. The yellow solution is poured into ice water and extracted with four 25-ml. portions of ether. The extract is washed with two portions of dilute sulfuric acid, two portions of sodium bicarbonate solution and water, then evaporated under nitrogen and lyophilized; yield 1.3 g. of viscous yellow oil (Note 7).

(d) *3,7,12,16-Tetramethyl-1,18-bis(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,-3, 5, 7, 11, 13, 15, 17-octadecaoctaen-9-yne-9, 10-C_2^{14}, (15, 15′- Dehydro-β-carotene-15,15′-C_2^{14})*. To a boiling solution of the crude C_{40}-ynediol in 80 ml. of sodium-dried toluene is added dropwise a hot solution of 0.025 g. of *p*-toluidinesulfonic acid (Note 8). Distillation is continued for 5 minutes; then the solution is cooled with an ice-bath, diluted with 100 ml. of petroleum ether, washed with sodium bicarbonate solution and water, and evaporated under vacuum. Purification is accomplished by dissolution of the crude product in 30 ml. of petroleum ether and chromatography on a 30 × 5-cm. column of magnesia-silica (1 : 1). The wide bright yellow product zone (Note 9) is eluted from the sectioned column with acetone, and the product is transferred to petroleum ether, washed to remove the acetone, dried over sodium sulfate, concentrated and crystallized from alcohol; yield 0.105 g. of reddish crystals with a metallic sheen (Note 9).

(e) *9, 10-mono-cis-3, 7, 12, 16-Tetramethyl-1,18-bis(2,6,6-trimethyl-1-cyclohexen-1-yl)- 1,3,5,7,9,11,13,15,17- octadecanonaene-9, 10-C_2^{14}, (15,15′-Mono-cis-β-carotene-15,15′-C_2^{14})*. The selective partial hydrogenation of the central triple bond is accomplished with a partially poisoned catalyst by the procedure of Inhoffen,[1,2] which follows. No attempt is made to crystallize the product.

The reaction is carried out in a photographic darkroom at least 2 meters from a carbon filament red safety light. A mixture of 0.220 g. of 15,15′-dehydro-β-carotene, 15 ml. of benzene, 0.200 g. of platinum-on-silica and 0.300 g. of platinum-on-carbon is stored for 12 hours; then the catalyst is filtered off and washed with 15 ml. of benzene. The combined filtrate is mixed with 0.015 g. of quinoline and 0.600 g. of palladium-on-calcium carbonate (Roche catalyst) and partially hydrogenated (Note 10). The catalyst is removed, and one-third of the filtrate is concentrated to 4–5 ml. at 0° and diluted with 12 ml. of methanol to crystallize the product. After three recrystallizations from benzene-methanol the yield is 0.030 g., m.p. 150°.

(f) *All-*trans*-3,7,12,16-Tetramethyl-1,18-bis(2,6,6-trimethyl-1-cyclohexen-1-yl)-1, 3, 5, 7, 9, 11, 13, 15, 17-octadecanonaene-9, 10-C_2^{14}, (All-*trans*-β-Carotene-15,15′-C_2^{14})*. The benzene solution (10 ml.) of 15,15′-mono-*cis*-β-carotene-15,15′-C_2^{14} is diluted with 1500 ml. of petroleum ether and exposed to diffuse sunlight in three filled, stoppered Erlenmeyer flasks. The isomerization is followed spectrophotometrically, the reaction being stopped when the ratio of the maximum at 450 mμ to that of the *cis*-peak at 338 mμ is about 10 : 1 (Note 11). The solution is concentrated to 100 ml. under vacuum and chromatographed, the chromatogram being developed with 1% acetone in petroleum ether. The product is adsorbed above the unreacted isomer and any dehydrocarotene. The latter two zones are recycled, and the product is recrystallized as in (d); yield 0.100 g., 10% based on the C_{19}-aldehyde (Note 12).

B. Notes

1. The procedure is an adaptation of that of Inhoffen.[2]

2. The preparation of the aldehyde is described by Inhoffen.[3]

3. The acetylene-C_2^{14} escaping from the mixture is captured as silver acetylide-C_2^{14} (to be recycled) in a trap containing ammoniacal silver nitrate and frozen with liquid nitrogen.

4. As the presence of water is detrimental, releasing acetylene, the ammonium chloride is dried under vacuum at 80°.

5. Absorption maximum, 280 mμ; a secondary absorption maximum at about 330 mμ is due to C_{19}-contaminant. The ratio of the two peaks is 3 : 1.

6. As the phenyllithium[4] is intended to be stoichiometrically sufficient to couple an additional 1.84 mmoles of C_{19}-aldehyde, it would appear that the amount of phenyllithium should be that equivalent to 0.026 g. (3.7 mmoles) of lithium metal. The carbinol group reacts with one equivalent of organolithium.

7. The absorption maximum in methanol is 280 mμ; m.p. 112.5° from benzene-petroleum ether.

8. The color changes from yellow to deep red as the acid is added.

9. Absorption maxima in petroleum ether, 433, 458 mμ, ratio 1.24 to 1; also see Inhoffen,[1,3] m.p. 155–156°. The zones are: I, narrow, light-yellow, presumably unreacted C_{19}-aldehyde; I a and b, fluorescent; II, product; III, strongly adsorbed, brownish-yellow.

10. One molecule of hydrogen is absorbed within 20 minutes, and the ultraviolet absorption spectrum in hexane possesses maxima at 449, and 477 mμ, with a *cis*-maximum at 338 mμ.

11. The ratio continues to rise on prolonged irradiation, but the absolute value at 450 mμ begins to fall, an observation to be avoided.

12. The radiochemical yield is 1.2%, absorption maxima 450, 478 mμ in hexane. *trans*-β-Carotene melts at 179–180°; the chromatography is described also by Karrer.[5]

[1]H. H. Inhoffen, F. Bohlmann and G. Rummett, Ann., *571*, 75 (1950).

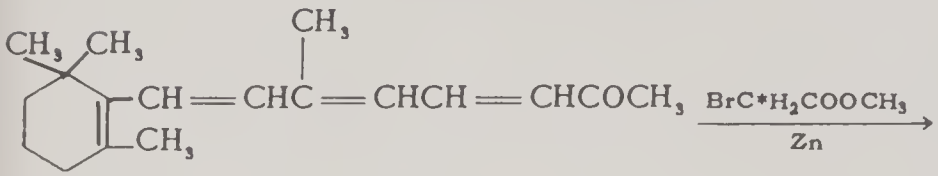
[2]H. H. Inhoffen, F. Bohlmann, G. Rummert and H. Pommer, Ann., *570*, 54 (1950); H. H. Inhoffen, U. Schwieter and G. Raspe, Ann., *588*, 117 (1954).

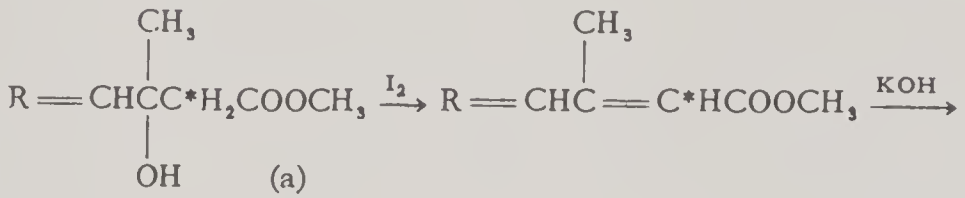
[3]H. H. Inhoffen and G. Leibner, Ann., *575*, 105 (1951).

[4]*Organic Syntheses*, Vol. 23, Wiley, New York, 1943, p. 83; *Organic Reactions*, Vol. VIII, Wiley, New York, 1954, p. 286.

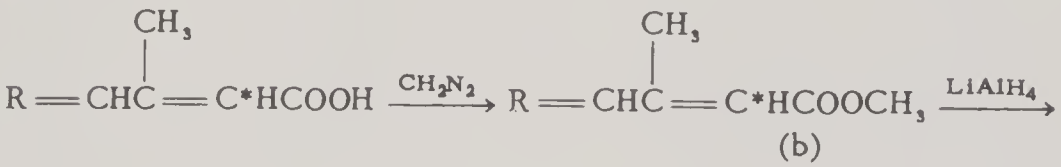
[5]P. Karrer and O. Walker, Helv. Chim. Acta, *16*, 641 (1933).

3,7-DIMETHYL-9-(2,6,6-TRIMETHYL-1-CYCLOHEXEN-1-YL)-2,4,6,8-NONATETRAEN-1-OL-2-C^{14} (C^{14}-Vitamin A_2)





(a)



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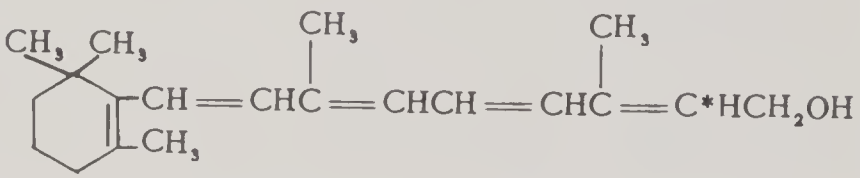


(c)

G. Wolf, B. C. Johnson and S. G. Kahn, *Radioisotope Conference, 1954*, Vol. I, Academic Press, New York, 1954, p. 283.

A. Procedure (Note 1)

(a) *Methyl 3-Hydroxy-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-4,6,8-nonatrienoate-2-C^{14}*. A stirred mixture of 0.3 mmole of 6-methyl-8-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3,5,7-octatrien-2-one (Note 2), 0.3 mmole of methyl bromoacetate and 3 mmoles of zinc is heated in dry benzene under dry helium (without reflux condenser). When the reaction has started, 2.7 mmoles of the ketone and 2.7 mmoles of methyl bromoacetate-2-C^{14} are added in benzene. The mixture is refluxed until reaction is complete (Note 3), then cooled and decomposed with cold 10% acetic acid. The product is extracted into petroleum ether (b.p. 90–100°) solution, which is washed with sodium bicarbonate and water and dried over sodium sulfate.

(b) *Methyl 3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoate-2-C^{14}*. The ester solution is refluxed for 5 minutes with

a small amount of iodine under helium (Note 4). The halogen is removed with sodium thiosulfate, and the solution is evaporated under reduced pressure. The residue is dissolved in petroleum ether (b.p. 40–60°) and placed on a column of acid-washed alumina (Note 5). A sharp yellow band is developed with petroleum ether and eluted with petroleum ether-benzene (6:4). Purification of the ester is accomplished by dissolution in ethanol and saponification for 3 days in the dark at room temperature with 25% potassium hydroxide. The alcohol is evaporated under vacuum, and the alkaline solution is washed with ether (Note 6) and adjusted to pH 3 with hydrochloric acid. The 3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic-2-C^{14} acid is collected by ether extraction and slowly crystallized as yellow needles from dry ether at 4°; yield 0.078 g. (8.7%), m.p. 174–176°; $\lambda_{max.}^{C_2H_5OH}$ 3470 A, $E_{1\ cm.}^{1\%}$ 1390 (Note 7).

The acid is quantitatively esterified with diazomethane in ether.

(c) *3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraen-1-ol-2-C^{14}.* To a solution of lithium aluminum hydride in absolute ether at −80° is added dropwise an ethereal solution of the ester. The mixture is maintained at −30° for 1 hour and decomposed by the addition of ethyl acetate, followed by water and sodium hydroxide to dissolve the aluminum hydroxide. The product is extracted into ether solution, which is washed with water under helium and evaporated under vacuum in the dark. The product is stored in petroleum ether solution in the presence of α-tocopherol. Paper chromatography (Note 8), radioautography and bio-assay indicate that the product is pure.

B. Notes

1. The procedure is an adaptation of the method of Wendler.[1]

2. The starting material may be prepared by treating 4-(β-ionylidene)-crotonic acid with methyllithium according to the procedure of Tegner[2] for preparing methyl ketones; other methods are described by Wendler.[1]

3. The color changes from light orange to reddish-brown; stirring is continued for about 1 hour.

4. Wendler[1] employed 0.050 g. of iodine at the 14-mmole scale, and 50 ml. of petroleum ether.

5. The method of separation is an adaptation of that of Garbers.[3]

6. The emulsion may be broken by centrifuging.

7. The molar specific activity is 107% of that of the starting material.

8. Filter paper impregnated with formamide is used; elution is with methanol-formamide (7 : 3) saturated with petroleum ether, R_f 0.7.

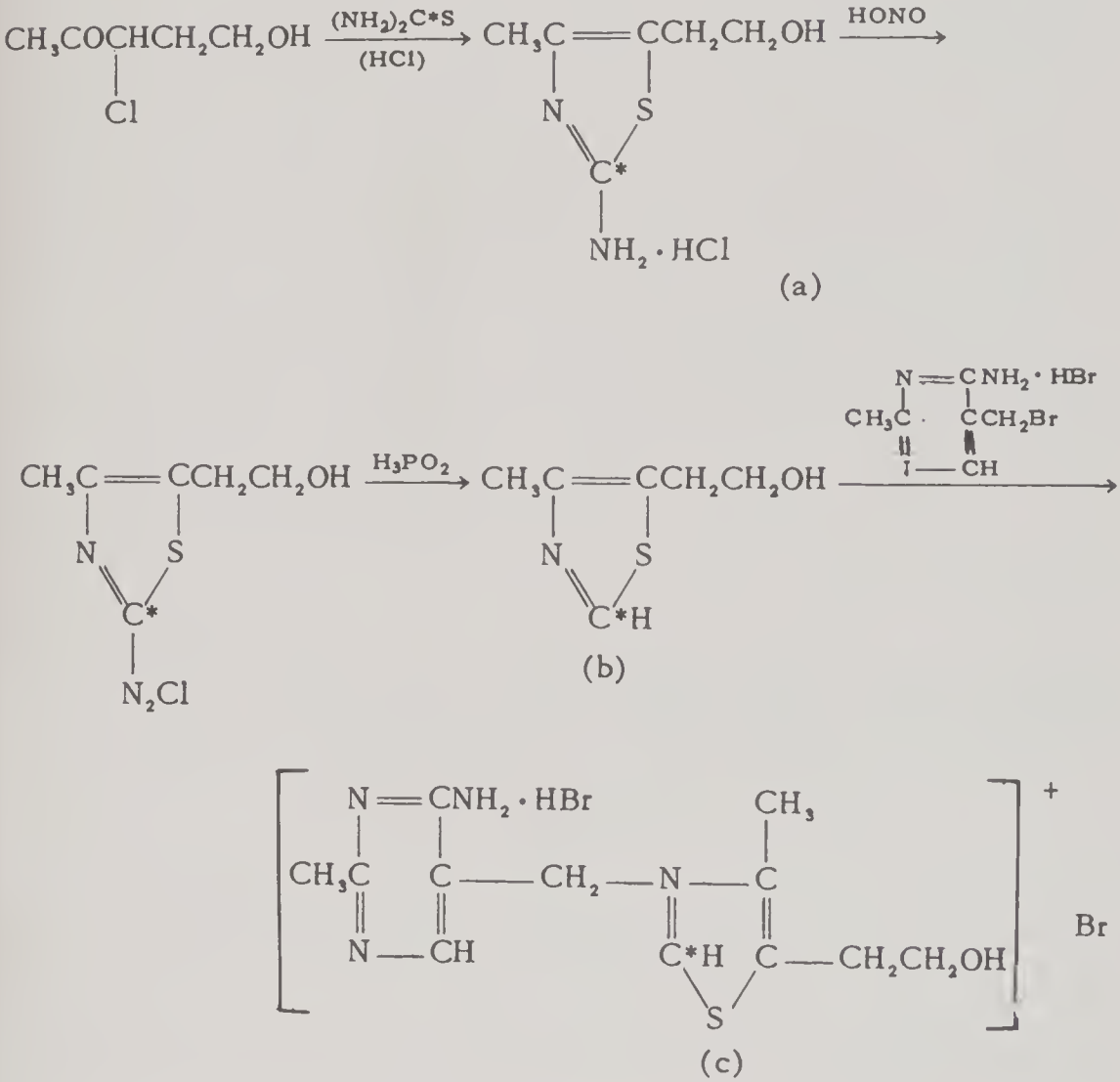
[1]N. L. Wendler, H. L. Slates, N. R. Trenner and M. Tischler, J. Am. Chem. Soc., 73, 719 (1951).

[2]C. Tegner, Acta Chem. Scand., 6, 782 (1952).

[3]C. F. Garbers, C. H. Eugster and P. Karrer, Helv. Chim. Acta, 36, 562 (1953).

3-(4-AMINO-2-METHYL-5-PYRIMIDINYLMETHYL)-5-(2-HYDROXYETHYL)-4-METHYLTHIAZOLIUM-2-C^{14} BROMIDE HYDROBROMIDE

(C^{14}-Vitamin B_1 Hydrobromide)



D. L. Williams and A. R. Ronzio, J. Am. Chem. Soc., 74, 2409 (1952); Atomic Energy Commission Report, AECU-1394; Nuc. Sci. Abstracts, 5, 5145 (1951).

A. Procedure

(a) *2-Amino-4-methyl-5-thiazoleëthanol-2-C^{14} Hydrochloride* (Note 1). To a refluxing aqueous solution of 0.1697 g. (2.2 mmoles) of thiourea-C^{14} is added dropwise 0.394 g. (2.9 mmoles) of 3-chloro-5-hydroxy-2-pentanone (Note 2). The mixture is heated for 2.5 hours, then acidified with 2 ml. of *N* hydrochloric acid and continuously extracted with ether for 8–10 hours. The aqueous solution is made strongly alkaline with 6 *N* sodium

hydroxide and extracted with ether for 48 hours. The extract is dried over Drierite (14 hours, Note 3), then concentrated to 50 ml., saturated with anhydrous hydrogen chloride, and cooled at 0° for 2 hours. The monohydrochloride is collected and dried under vacuum; yield 0.3746 g. (86.7%), m.p. 153–154°.

(b) *4-Methyl-5-thiazoleëthanol-2-C^{14}* (Note 4). The 2-amino-4-methyl-5-thiazoleëthanol-2-C^{14} hydrochloride (1.93 mmoles) is dissolved in 9.6 ml. of concentrated hydrochloric acid with cooling at −5°. To the stirred cold solution is added 2.9 ml. of ice-cold *N* sodium nitrite solution during 15 minutes. The mixture is stored at −5° for 30 minutes, then slowly diluted with 9.6 ml. of ice water to dissolve the colorless precipitate. To the stirred diazonium salt solution, maintained at −5°, is added 4.2 ml. (2.3 mmoles) of 32% hypophosphorous acid during 13 minutes. The mixture is stored in a refrigerator at 2–4° until the evolution of nitrogen is complete (16 hours); then the cooled solution (−5°) is adjusted slowly to pH 10–12 with 6 *N* sodium hydroxide and extracted with ether for 40 hours. The extract is dried over 4.5 g. of magnesium sulfate for 48 hours, and the solution is filtered into a 50-ml. cone-shaped flask, the drying agent being washed with five 20-ml. portions of ether and the solution concentrated to 1–2 ml. (Note 5).

(c) *3-(4-Amino-2-methyl-5-pyrimidinylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium-2-C^{14} Bromide Hydrobromide, (C_1^{14}-Thiamin Bromide Hydrobromide)* (Note 6). To a mixture of 1 ml. of butanol and the thiazole solution, heated at 125° under reflux, is added 0.382 g. (1.34 mmoles) of 4-amino-5-(bromomethyl)-2-methylpyrimidine hydrobromide in small portions over 20 minutes (Note 7). The mixture is heated for 15 minutes, then stored at room temperature for 15 hours. The crystalline product is collected, washed with 0.5 ml. of absolute alcohol and two 2-ml. portions of absolute ether, then dried in a vacuum desiccator; yield 0.3341 g., 50% based on thiazole. A solution of the crude product in 2 ml. of hot methanol is diluted with 8 ml. of warm butanol, cooled slowly and stored in a refrigerator for 24 hours to crystallize; yield 26.6% based on thiazole (Note 8).

B. Notes

1. The procedure is a modification of the method of Hatcher.[1]

2. 2-Acetobutyrolactone, prepared by the procedure of Knunyantz,[2] is chlorinated with sulfuryl chloride and converted to the desired starting material by the method of Buchman.[3]

3. Since the 2-amino-4-methyl-5-thiazoleëthanol-2-C^{14} is only slightly soluble in ether, any compound adsorbed on the drying agent (up to 14%)

must be removed by extraction in a Soxhlet apparatus. The free base melts at 93°, after recrystallization from benzene, and the monopicrate melts at 218–219°.

4. The procedure is an adaptation of the method of Hatcher,[1] and was arrived at after consideration of a number of alternative deamination methods. The optimum yield of 70% is obtained reproducibly when the diazotization is effected in concentrated hydrochloric acid, followed by the reduction in *6N* hydrochloric acid with 12 molar amounts of 32% hypophosphorous acid.

5. The picrate, m.p. 162–163°, is very slightly soluble in dry ether.

6. The procedure is an adaptation of the method of Cline,[4] modified to conserve the thiazole. While an excess of the thiazole is conducive to high yields of thiamin,[5] because of a side reaction involving the reaction of the 5-(bromomethyl) group of the pyrimidine with carbinols to form ethers, the incremental addition of an equimolar amount of the pyrimidine achieves the same effect. There is no unreacted thiazole present, and the pyrimidine reacts with the thiazole to form products other than thiamin. An excess of the pyrimidine appears to reduce the yield of thiamin, as does the use of inert solvents.

7. Following the last addition, the condenser is washed down with 0.5 ml. of absolute alcohol.

8. The compound melts at 238–239° when crystallized from methanol-butanol, and at 223–224° when crystallized from methanol. Authentic samples melted at 239–240° and 226–227°, respectively. The yield in five trial runs was 40.7–70.9% (crude) and 27.5–44% (purified).

[1]J. B. Hatcher, J. Am. Chem. Soc., 69, 465 (1947).

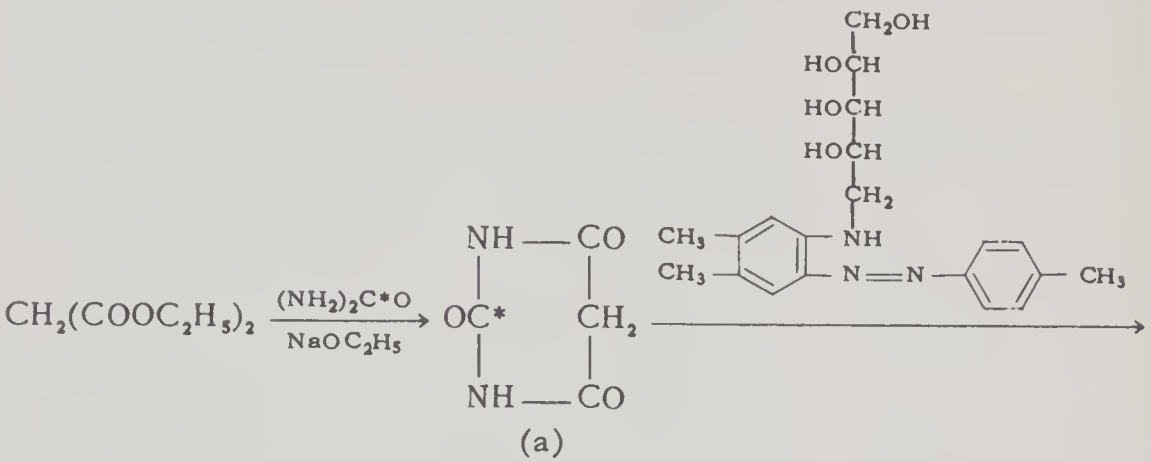
[2]I. L. Knunyantz, G. V. Chelintzev and E. D. Osetrova, Compt. rend. acad. sci., (URSS) [N.S.], *1*, 315 (1934); through Chem. Abstracts, *28*, 4382 (1934).

[3]E. R. Buchman, J. Am. Chem. Soc., *58*, 1803 (1936).

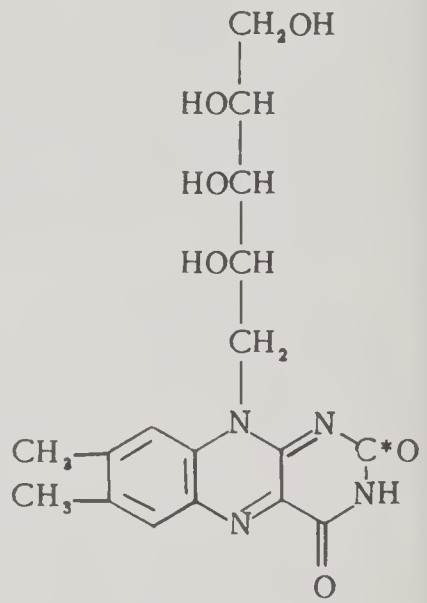
[4]J. K. Cline, R. R. Williams and J. Finkelstein, J. Am. Chem. Soc., *59*, 1052 (1937).

[5]P. Baumgarten, A. Dornow, K. Gutschmidt and H. Krehl, Ber., *75B*, 444 (1942).

7,8-DIMETHYL-10-(D-RIBITYL)ISOALLOXAZINE-2-C^{14} (C_1^{14}-Vitamin B_2)



(a)



(b)

E. E. Haley and J. P. Lambooy, J. Am. Chem. Soc., 76, 2926 (1954).

A. Procedure

(a) *Barbituric-2-C^{14} Acid*, (*Malonylurea-C^{14}*) (Note 1). To a warm, stirred solution of 0.231 g. of urea-C^{14}, in a solution of 0.10 g. of sodium in 2 ml. of absolute ethanol, is added 0.62 ml. of ethyl malonate in 1.0 ml. of absolute alcohol. The mixture is heated at 108° for 7 hours in a sealed tube, 1.5 × 13 cm. A solution of the reaction mixture in 17 ml. of hot water is acidified with 1.0 ml. of concentrated hydrochloric acid and stored in a refrigerator to crystallize; yield 0.374 g., 76% (Note 2). The radiochemical yield is 82%.

(b) *7,8-Dimethyl-10-(D-ribityl)isoalloxazine-2-C^{14}*, (*D-Riboflavin-2-C^{14}*). Barbituric-2-C^{14} acid (9.374 g.) is condensed with 1.09 g. (Note 3) of *N*-

(D-ribityl)-6-(*p*-tolylazo)-3,4-xylidine (Note 4), according to a suitable adaptation of the method of Tishler,[1] which follows. The product is purified by recrystallization from hot water; yield 0.504 g., 46% (Note 5).

A mixture of 27 g. of 6-(*p*-nitrophenylazo)-*N*-(D-ribitylamino)-3,4-xylidene, 14.3 g. of barbituric acid, 180 ml. of dioxane and 34 ml. of acetic acid is refluxed for 5 hours (Note 6). The mixture is cooled, and the crude product is collected, washed with hot water and dried; yield 21.0 g. Purification is accomplished by dissolution in 4 volumes of 18% hydrochloric acid, decolorizing with a few drops of Superoxol, filtering and diluting with water; yield 16.5 g., 65.8%.

B. Notes

1. The procedure is an adaptation of the method of Slimmer.[2] Also see sodium 5-ethyl-5-(2-methylbutyl)barbiturate-2-C^{14}.

2. The compound crystallizes from water as a dihydrate; drying for 3 hours at 105° forms the anhydrous acid, m.p. 248°.

3. Trial experiments have shown that the yield of product is not appreciably diminished when equimolar amounts of reactants are used.

4. D-Ribose is condensed[3] with 3,4-xylidine,[4] and the 3,4-xylidine-*N*-D-ribopyranoside is hydrogenated over palladium-on-calcium carbonate for 3 hours at 60 p.s.i. and 70°. The *N*-(D-ribityl)-3,4-xylidine is converted[1] to the starting reagent by coupling with *p*-toluenediazonium chloride. The resulting *N*-(D-ribityl)-6-(*p*-tolylazo)-3,4-xylidine (m.p. 171–172°) is contaminated with appreciable amounts of the 2-arylazo isomer which, however, does not react with barbituric acid.

5. The radiochemical yield is 41%. The purity is established by microbiological assay,[5] paper chromatography and radioautography. The chromatogram is developed in butyl alcohol-water-acetic acid (4 : 5 : 1).

6. Weak acids catalyze the reaction. Refluxing is continued until the azo compound is consumed, as demonstrated by the failure of an aliquot to develop a deep color with concentrated hydrochloric acid.

C. Other Preparations

The preparation of barbituric-2-C^{14} acid by the method of Biltz[6] has been described by Tamayo.[7] To a stirred solution of 13 g. of malonic acid and 6.5428 g. of urea-C^{14} in 26 ml. of glacial acetic acid on a water-bath is added 26 ml. of acetic anhydride dropwise during 25 minutes. The mixture is heated at 75–80° for 3 hours and at 90° for 3 hours, then cooled with an ice-bath, and the product is collected, recrystallized from water and dried; yield 8.77 g. of anhydrous product, m.p. 245°.

[1]M. Tishler, K. Pfister, 3rd, R. D. Babson, K. Ladenburg and A. J. Fleming, J. Am. Chem. Soc., 69, 1487 (1947)

[2]M. Slimmer and J. Stieglitz, Am. Chem. J., *31*, 677 (1904).
[3]L. Berger and J. Lee, J. Org. Chem., *11*, 84 (1946).
[4]*Organic Syntheses*, Vol. 28, Wiley, New York, 1948, p. 22.
[5]O. Rahn and B. Iske, Growth, *15*, 147 (1951).
[6]H. Biltz and H. Wittek, Ber., *54*, 1035 (1921).
[7]M. L. Tamayo, J. M. Gamboa and E. F. Alvarez, Anales real soc. españ. féz. y quím, *48B*, 889 (1952); through Chem. Abstracts, *48*, 1278 (1954).

CYANO-C^{14}-COBALAMIN
(C_1^{14}-Vitamin B_{12})

$$\text{Vitamin } B_{12a} \xrightarrow{HC^*N} \text{Vitamin } B_{12}$$

G. E. Boxer, J. C. Rickards, C. Rosenblum and D. T. Woodbury, Arch. Biochem., *30*, 470 (1951).

A. Procedure

Hydroxocobalamin (Note 1) is converted to vitamin B_{12} by treatment with hydrogen cyanide-C^{14} and is crystallized from acetone. The procedure for a similar preparation is given by Kaczka,[1] as follows.

To 2 ml. of aqueous solution containing 0.0037 g. of hydroxocobalamin (Vitamin B_{12a}) is added 0.5 ml. of a solution containing 0.0066 g. of potassium cyanide, and the mixture is adjusted to pH 5–6 with dilute hydrochloric acid. The product is extracted with two 1.5-ml. portions of phenol-carbon tetrachloride (1 : 7 v/v), equilibrated with water. The combined extract is washed with four 1-ml. portions of water, previously equilibrated with the phenol-carbon tetrachloride solution. The phenol-carbon tetrachloride solution is then diluted with 12 ml. of carbon tetrachloride, and the product is extracted with two 1-ml. portions of water. The combined aqueous solution is washed with ether and evaporated to dryness under vacuum at room temperature. The residue is dissolved in 0.5 ml. of water, and the solution is diluted with 12 ml. of acetone and set aside to crystallize. The slender, red needles are collected by centrifuging, washed with acetone and dried; yield 0.0023 g. (Note 2).

B. Notes

1. The starting material is easily prepared by irradiating an aerated solution of cyanocobalamine, or vitamin B_{12}, at room temperature with visible artificial light by the procedure of Boxer[2]. The liberation of the cyano group, coordinatively bound to cobalt, is quantitative. The structure of the compound (molecular weight about 1400) is not yet fully known.

2. The homogeneity of the product was established by an eight-stage countercurrent distribution analysis,[3] with ultraviolet absorption and

chemical and radiochemical determination of bound cyanide[4] in each of the sixteen fractions. It has been demonstrated[5] that crystalline vitamin B_{12} does not exchange with cobalt in acidic, neutral or alkaline solutions, even after 3 months.

[1]E. A. Kaczka, D. E. Wolf, F. A. Kuehl, Jr., and K. Folkers, Science, *112*, 354 (1950).

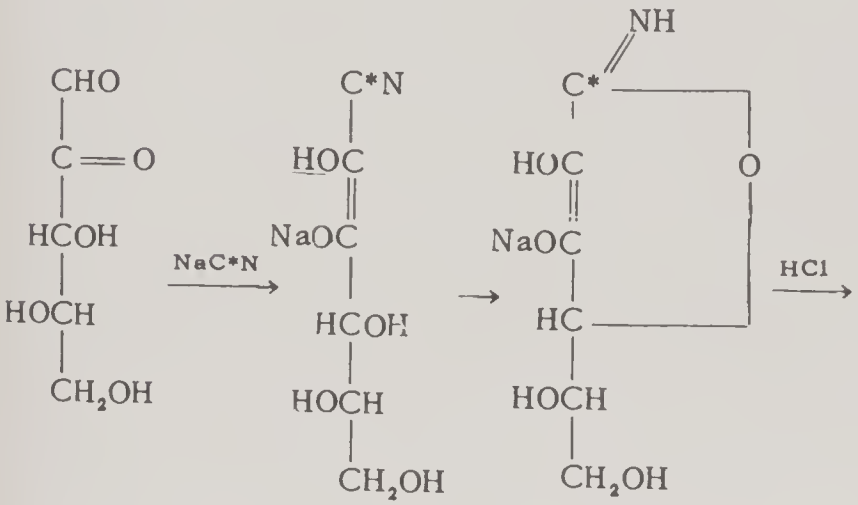
[2]G. E. Boxer and J. C. Rickards, Arch. Biochem., *30*, 382 (1951).

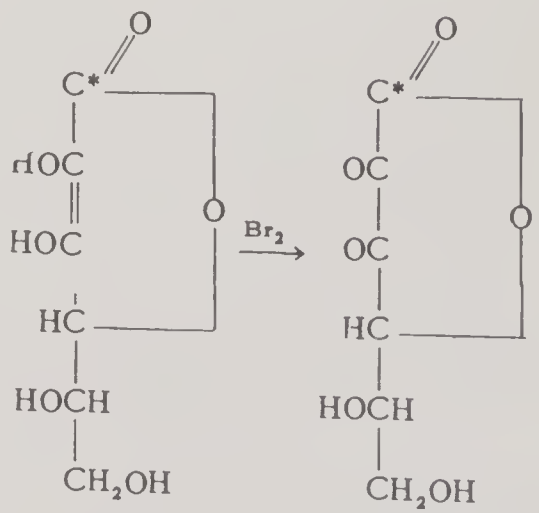
[3]L. Chaiet, C. Rosenblum, and D. R. Woodbury, Science, *111*, 601 (1950).

[4]G. E. Boxer and J. C. Rickards, Arch. Biochem., *30*, 372, 382 (1951).

[5]R. R. Baldwin, J. R. Lowry and R. V. Harrington, J. Am. Chem. Soc., *73*, 4968 (1951); R. N. Boos, C. Rosenblum and D. T. Woodbury, J. Am. Chem. Soc., *73*, 5446 (1951).

L-ASCORBIC-1-C^{14} ACID
(C_1^{14}-Vitamin C)





L. L. Salomon, J. J. Burns and C. G. King, J. Am. Chem. Soc., *74*, 5161 (1952); Science, *111*, 257 (1950).

A. Procedure (Note 1)

A solution of 0.600 g. (4.0 mmoles) of L-xylosone (Note 2) and 15 ml. of water is added dropwise (20 minutes), in a nitrogen atmosphere, to 15 ml. of a stirred solution containing 3 mmoles of sodium cyanide-C^{14} and 6 mmoles of sodium hydroxide, in a 125-ml. Erlenmeyer flask which may be sealed by means of stopcocks. A slow stream of nitrogen is passed through the flask into a trap containing 50 ml. of 2.5 *N* sodium hydroxide, and the reaction mixture is stirred for 10 minutes (Note 3). The imino-L-ascorbic-1-C^{14} acid mixture is made 2.5 *N* in acid by addition of concentrated hydrochloric acid. Carrier ascorbic acid (0.600 g.) is added and the flask is sealed and heated for 24 hours in a water-bath at 50°. The flask is swept with nitrogen to remove carbon-C^{14} dioxide (Note 4); then the mixture is evaporated under reduced pressure at 30°. The residue is taken up in 100 ml. of water and passed through an anion exchange column in the acetate form (Note 5). The column is eluted with *N* hydrochloric acid, and those fractions containing product, determined by titration with indophenol, are collected under nitrogen and evaporated at 30° under vacuum. A solution of the residue in 30 ml. of absolute alcohol is diluted with 400 ml. of absolute ether (peroxide-free), filtered and evaporated under nitrogen. The crystalline product is washed with absolute ethanol-ligroin (redistilled) (1:1), until the liquor is no longer colored, then once with absolute ether; yield 0.667 g., m.p. 187–189° (Note 6). The radiochemical yield is 58% based on sodium cyanide; sufficient additional product may be obtained from the residues by the use of carrier to raise the yield to 70%.

B. Notes

1. The procedure is a modification of the methods of Ault[1] and Reichstein[2] and parallels the method of Hamilton,[3] who has improved the yield by carrying out the hydrolysis at 95–100° for 3 hours.

2. Directions are given by Salomon for preparing the starting material in good yield from D-sorbitol, or L-xylose.

3. Titration of the product (in trial runs) with indophenol dye, standardized against ascorbic acid, shows that the addition of cyanide is quantitative. The titration may also be performed with iodine.[3]

4. As much as 15–20% ascorbic acid appears to be lost by decarboxylation during hydrolysis. The carbon dioxide is trapped in saturated barium hydroxide solution.

5. A 2 × 50-cm. column containing a 30-cm. bed of Amberlite IR-4B is conditioned by the method of Jackel,[4] then converted to the acetate form

by upflow with 10% acetic acid, washed with water and saturated with nonisotopic ascorbic acid, then eluted with 2 *N* ammonium hydroxide and reconverted to the acetate form. The use of the anion column in the acetate, rather than the base form, obviates the need for the prior use of a cation column.

6. The product is pure, as measured by titration. The molar specific activity does not change upon recrystallization from glacial acetic acid, nor upon conversion to the isopropylidene derivative.

C. Other Preparations

Mosbach[5] has demonstrated that when L-ascorbic-1-C^{14} acid reacts with formaldehyde, the carbon dioxide evolved comes exclusively from the carboxyl group; the product is not yet identified.

Dehydroascorbic-1-C^{14} acid, m.p. 225° (dec.), is prepared from L-ascorbic-1-C^{14} acid by oxidation with bromine[6] or with an equivalent of quinone.[7] In the latter case the freshly prepared solution contained less than 2% of the L-ascorbic-1-C^{14} acid, as shown by paper chromatography and spectrophotometric analysis.[8]

The conversion of L-ascorbic-1-C^{14} acid to 2,3-dioxo-L-gulonic-1-C^{14} acid or calcium 2,3-dioxo-L-gulonate-1-C^{14} by a modification of the method of Penney[9] has been described by Curtin,[7] and reported by Burns;[6] yield of octahydrate 66%, $[\alpha]_D^{20}$ −8.6°. The molar specific activity was the same as that of the starting material. A single radioactive spot was obtained by paper chromatography,[8] employing butanol-acetic acid-water, isobutyric acid-water, and butanol-ethanol-water.

[1]R. G. Ault, D. K. Baird, H. C. Carrington, W. N. Haworth, R. Herbert, E. L. Hirst, E. G. V. Percival, F. Amith and M. Stacey, J. Chem. Soc., *1933*, 1419.

[2]T. Reichstein, A. Grussner and R. Oppenauer, Helv. Chim. Acta, *16*, 1019 (1933); *17*, 510 (1934).

[3]J. K. Hamilton and F. Smith, J. Am. Chem. Soc., *74*, 5162 (1952).

[4]S. S. Jackel, E. H. Mosbach and C. G. King, Arch. Biochem., *31*, 442 (1951).

[5]E. H. Mosbach, J. J. Burns and C. G. King, J. Am. Chem. Soc., *73*, 1874 (1951).

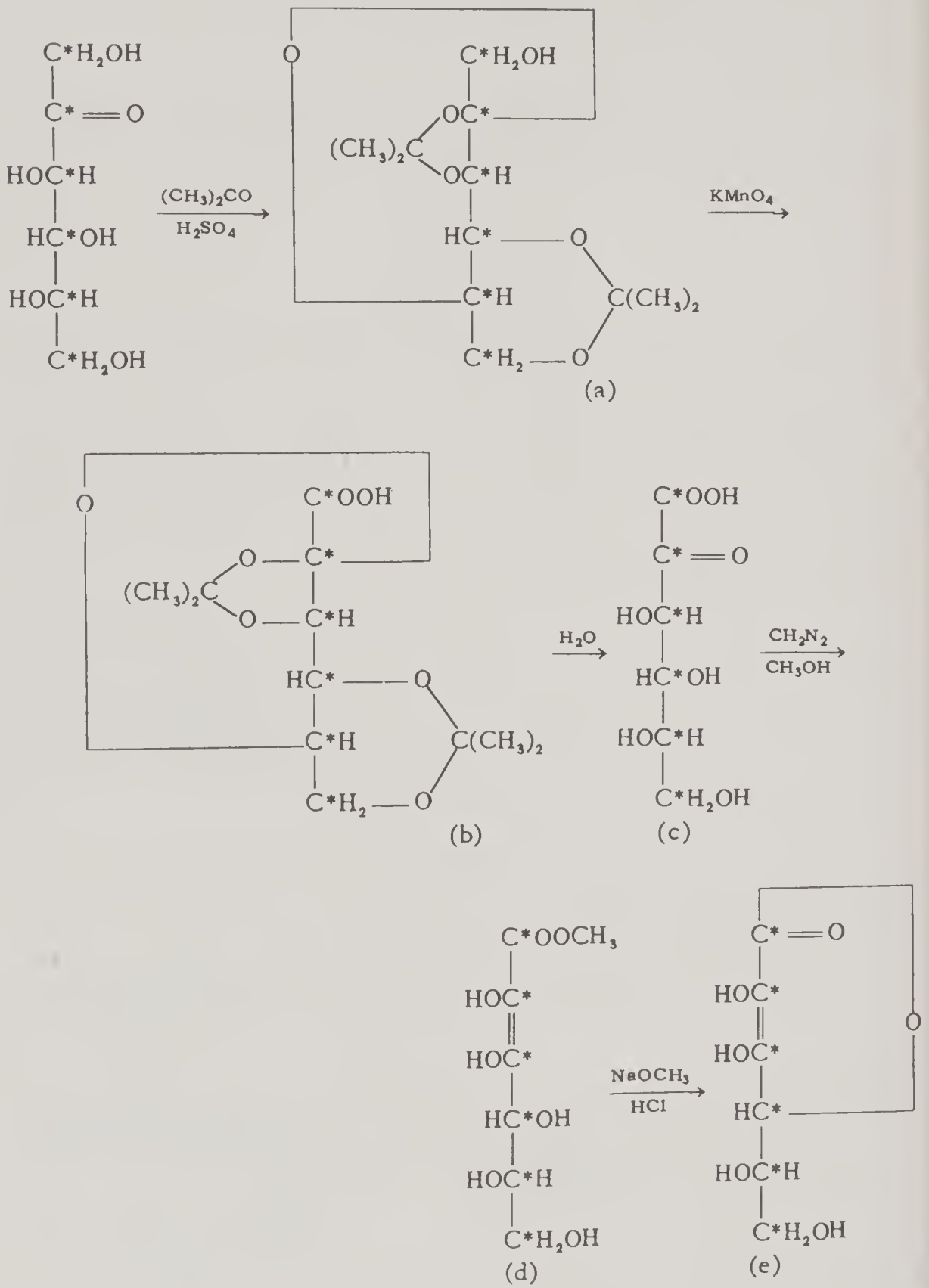
[6]J. J. Burns, E. H. Mosbach and S. Schulenberg, J. Biol. Chem., *207*, 679 (1954).

[7]C. O'H. Curtin and C. G. King, J. Biol. Chem., *216*, 539 (1955); M. O. Schultze, C. J. Harrer and C. G. King, J. Biol. Chem., *131*, 5 (1939).

[8]Univ. Texas Publ., No. 5109 (1951).

[9]J. R. Penney and S. S. Zilva, Biochem. J., *39*, 1 (1945).

L-ASCORBIC-C_6^{14} ACID



A. A. Bothner-By, M. Gibbs and R. C. Anderson, Science, *112*, 363 (1950).

A. Procedure (Note 1)

(a) *2,3:4,6-Di-O-isopropylidene-L-sorbose-C_6^{14}*. L-Sorbose-C_6^{14} (0.479 g., Note 2) is acetonated in freshly distilled dry acetone, and the product is separated by ether extraction according to the procedure of Reichstein,[1] which follows.

A mixture of 100 g. of sorbose, 2 l. of acetone and 80 ml. of concentrated sulfuric acid is shaken for just 20 hours. The vigorously stirred mixture is cooled and slowly neutralized with 300 g. of powdered potassium carbonate during about 5 hours. The solution is filtered from the salt cake, the solvent is evaporated, and the residue is extracted with ether. The extract is washed with 20% potassium carbonate solution, dried and concentrated, and the product is vacuum-distilled (135° at 0.3 mm.). The residue consists of 45 g. of monoacetone-L-sorbose, which may be converted by further treatment. The yield is 75 g., m.p. 77–78°, after crystallization from petrol (Note 3).

(b) *2,3:4,6-Di-O-isopropylidene-2-keto-L-gulonic-C_6^{14} Acid* (Note 4). The diacetone derivative is oxidized in 5% potassium hydroxide solution at 18–20° by the slow addition of 6% potassium permanganate solution with vigorous stirring. The mixture is stirred for 4 hours and filtered, and the residue is thoroughly washed with hot water. Unreacted starting material, recovered by ether extraction of the combined solutions, is submitted to reoxidation. The cooled alkaline solution is acidified to pH 2 with hydrochloric acid, and the product is extracted into ethyl acetate (Note 5).

(c) *2-Keto-L-gulonic-C_6^{14} Acid.* The diisopropylidine-2-keto-L-gulonic-C_6^{14} acid is refluxed with water for 40 minutes. Lyophilization gives a white powdery product, m.p. 170–171°.

(d) *Methyl 2-Keto-L-gulonate-C_6^{14}*. The 2-keto-L-gulonic-C_6^{14} acid is esterified in methanol solution at –15° by addition of a large excess of diazomethane. The solvent and excess reagent are removed by evaporation, and the product is crystallized from methanol-acetone. The first crop yield is 0.068 g., m.p. 145–150° (Note 6). The 0.220 g. of residual oil obtained from the mother liquor is treated with 0.205 g. of carrier for recovery of product, which is worked up separately.

(e) *L-Ascorbic-C_6^{14} Acid.* The methyl 2-keto-L-gulonate-C_6^{14} is treated in anhydrous methanol solution, under a nitrogen atmosphere (Note 8), with the stoichiometric amount of sodium methoxide. The mixture is boiled for 1 minute, cooled, and treated with the stoichiometric amount of methanolic hydrogen chloride. The product is purified according to the method of Szent-Gyorgyi[2] (Note 9). The yield is 0.030 g. (plus 0.070 g. obtained with the use of carrier), m.p. 189–190°; the latter product has a specific activity equal to 23% of the former. Paper chromatography shows the presence of only one compound (Note 10).

B. Notes

1. The procedure is adapted from the method of Reichstein.[1] Also see Strukov.[1a]

2. Uniformly labeled glucose (0.392 g.) in aqueous alkali is hydrogenated over Raney nickel. The D-sorbitol is oxidized biochemically to L-sorbose[3] by fermentation, and the desired starting material is isolated through the use of ion exchange resins and crystallization; yield 0.344 g., m.p. 163–165°

3. Anhydrous copper sulfate may be used as catalyst;[4] the yield on the 200-g. scale is 90–92%.

4. The procedure is adapted from the method of Maksimov.[4]

5. The product forms a hydrate,[1] m.p. 86–98°, yield 90%.

6. The pure ester melts at 155–157°, and the yield is about 18 g. from 20 g. of keto acid.

7. On the usual laboratory scale the yield[1] is about 84% crude, and 73% after purification, m.p. 189–190° (dec.).

8. Ascorbic acid is oxidized in aqueous solution by contact with air.

9. The acid is precipitated from aqueous or alcoholic solution by basic lead acetate. The salt may be decomposed in dry acetone suspension by the action of hydrogen sulfide, and the filtrate is treated with ether and petroleum ether to precipitate the ascorbic acid.

10. The stepwise degradation of ascorbic acid has been described by Horowitz.[5]

[1]T. Reichstein and A. Grussner, Helv. Chim. Acta, *17*, 311 (1934); T. Reichstein, U.S. 2,265,121; Chem. Abstracts, 36, 1739, (1942).

[1a]I. T. Strukov and N. A. Kopylova, Farmatsiya *10* (3), 8 (1947); through Chem. Abstracts, *44*, 8327 (1950).

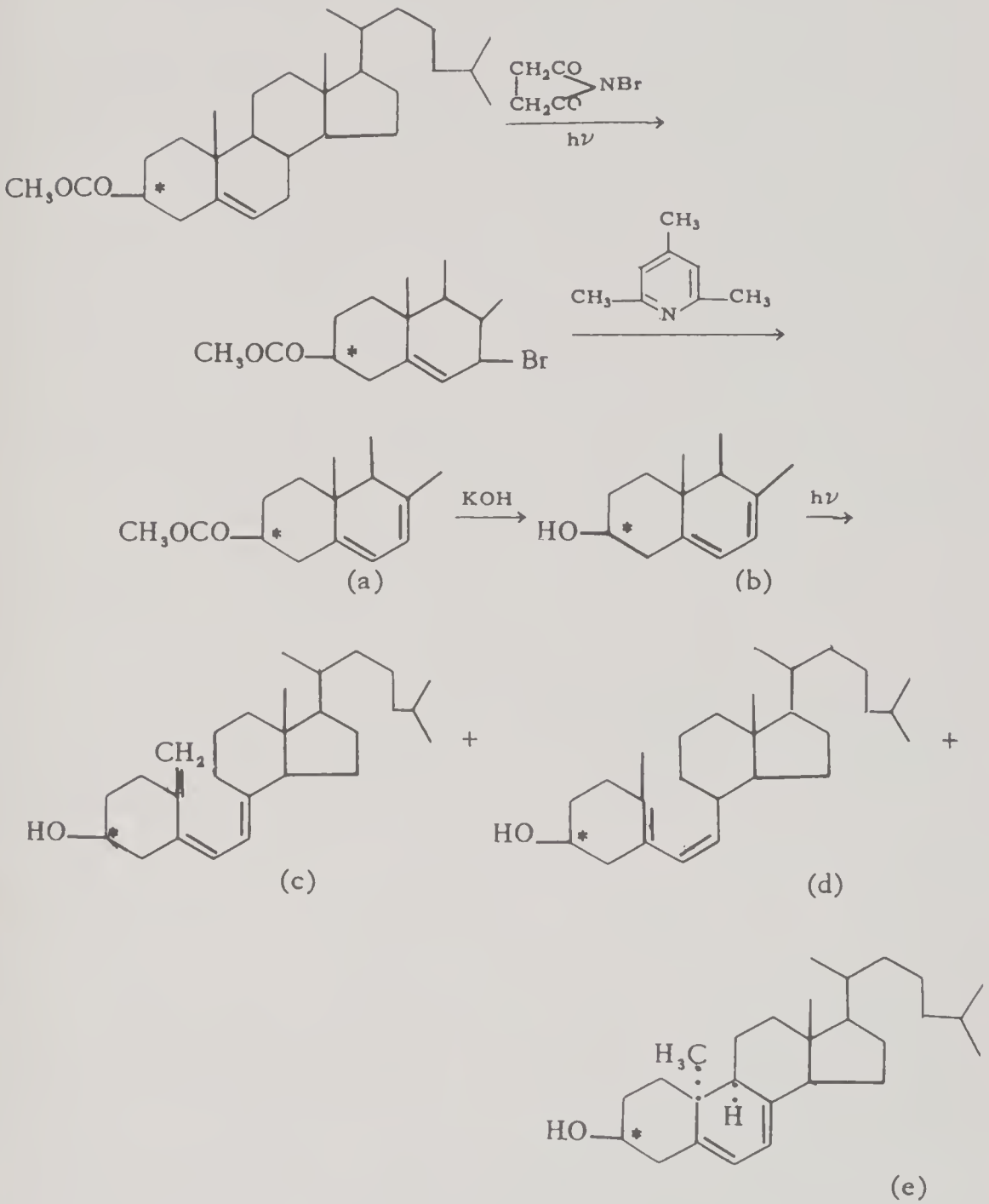
[2]A. Szent-Györgyi, Biochem. J., *22*, 1394 (1928).

[3]P. A. Wells, J. J. Stubs, L. E. Lockwood and E. T. Roe, Ind. Eng. Chem., *29*, 1385 (1937).

[4]V. I. Maksimov, V. V. Nikonova, A. F. Lozarev and L. A. Zvereva, J. Gen. Chem. (U.S.S.R), *9*, 936 (1939); through Chem. Abstracts, *34*, 380 (1940).

[5]H. H. Horowitz and C. G. King, J. Biol. Chem., *200*, 125 (1953); H. H. Horowitz, A. P. Doerschuk and C. G. King, J. Biol. Chem., *199*, 125 (1952).

VITAMIN D_3-3-C^{14}



E. Havinga and J. P: L. Bots, Rec. trav. chim., 73, 393 (1954).

A. Procedure

5,7-Cholestadien-3β-ol-3-C^{14}, (*7-Dehydrocholesterol-3-*C^{14}). Cholesteryl-3-C^{14} acetate (0.300 g.) is converted to the product (40% yield) by

the procedure of Buisman,[1] which follows. Purification is accomplished by conversion to the dinitrobenzoate, then recrystallization of this ester, followed by saponification; yield 0.120 g., purity 89% (Note 1).

(a) *5,7-Cholestadien-3β-yl-3-C*^{14} *Acetate,* (*7-Dehydrocholesteryl-3-C*^{14} *Acetate*). A stirred mixture of 21.4 g. (50 mmoles) of cholesteryl acetate, 13 g. (73 mmoles) of *N*-bromosuccinimide and 330 ml. of petroleum ether (b.p. 65–75°) is refluxed for 7 minutes under illumination from a mercury lamp. The precipitate is separated from the cooled solution and washed with light petroleum. To the filtrate containing 7-bromocholestryl-3-C^{14} acetate is added 10 ml. of *s*-collidine; then the petroleum ether is evaporated under reduced pressure, and the residue is dissolved in 100 ml. of xylene. The solution is heated under nitrogen with 5 g. of calcium carbonate for 20 minutes at 125°. The cooled solution is filtered, and the xylene and collidine are removed by steam distillation under reduced pressure (nitrogen atmosphere). The flask is cooled, and the water is decanted from the residual yellow cake, which is washed with alcohol and recrystallized from warm acetone; yield 6.1 g., 29% (Note 2).

(b) *5,7-Cholestadien-3β-ol-3-C*^{14}, (*7-Dehydrocholesterol-3-C*^{14}). The ester is hydrolyzed by treating a benzene solution with methanolic potassium hydroxide and storing the solution overnight at room temperature. The product crystallizes in long needles, m.p. 149.0–149.5° (in vac.) after recrystallization from methanol.

(c) *Vitamin* D_3*-3-C*^{14} (Note 3). The apparatus consists of a quartz irradiation vessel, comprised of three concentric compartments surrounding a 250-W mercury lamp (Note 4). Cooling water circulates in the inner compartment; the second contains 0.4% aqueous sodium nitrate (Note 5); the reaction solution is circulated through the third by means of a rotating glass spiral impeller (Note 6). Stopcocks permit evacuation of the space above the reaction solution and filling with nitrogen.

A solution of 0.120 g. of 5,7-cholestadien-3β-ol-3-C^{14} in 135 ml. of peroxide-free ether is irradiated for 7.5 minutes. The solvent is replaced by alcohol; then the solution is treated with digitonin in 80% alcohol to percipitate the unreacted 7-dehydrocholesterol-3-C^{14} (Note 7). The filtrate is diluted with 150 ml. of ether, and washed with a mixture of 180 ml. of saturated sodium chloride and 180 ml. of water to remove the excess digitonin. The ether solution is concentrated, and the residue is dried azeotropically with absolute benzene-absolute alcohol (1:1), then with dry benzene under vacuum at low temperature (Note 8). The residue is dissolved in 10 ml. of benzene, and the solution is heated with 1 ml. of 10% maleic anhydride in benzene for 30 minutes at 75° under carbon dioxide. The solvent is evaporated under reduced pressure, and the residue is mixed with 10 ml. of 2 *N* potassium hydroxide in methanol. After 20 minutes at room temperature, the solution is diluted with 20 ml.

of water and extracted with ether (Note 9). The extract is concentrated, and the residue is dried azeotropically, as above, and dissolved in 2 ml. of dry benzene. The solution is mixed with 1 ml. of dry pyridine and 2 ml. of 20% solution of 3,5-dinitrobenzoyl chloride in benzene. The mixture is stored at room temperature for 30 minutes; then the benzene is distilled. The residue is mixed with 5 ml. of pyridine and 20 ml. of water, and the solution is extracted with ether. The extract is washed with water, concentrated and dried azeotropically with 2-butanone. The residue is dissolved in 10 ml. of 8% ether in petroleum ether (40–60°), and the filtered solution (Note 10) is separated by chromatography on 10 g. of neutral alumina (Brockmann activity III)[2] and eluted with the same solvent (50 ml. total). The solvent is evaporated, and the residue (Note 11) is dissolved in 2 ml. of dioxane and treated with 2 ml. of 2 *N* methanolic potassium hydroxide solution. The mixture is stored for 30 minutes at room temperature, then diluted with 20 ml. of water and extracted with ether. The extract is washed with water and concentrated, and the residue is dried azeotropically with cyclohexane; yield 0.0174 g. (Note 12).

B. Notes

1. Ultraviolet absorption data indicates that the major impurities are cholesterol and 0.7% 4,6-cholestadien-3-ol. Cholesterol does not interfere in the following step. Pure 7-dehydrocholesteryl 3,5-dinitrobenzoate melts[3] at 205–207°.

2. Pure 7-dehydrocholesteryl acetate melts at 130.0–130.5° (under vacuum).

3. The procedure is essentially that of Buisman;[1] and of Havinga, Koevoet and Verloop.[4,5]

4. A diagram of the apparatus is presented by Havinga and Bots, Figure XIV, 1. The lamp (Biosol A) is screened by a copper tube, except for a 1-cm. opening. The lamp is raised up into an iron case above the irradiation vessel and burned for 15 minutes, prior to lowering into operating position, in order to attain constant and reproducible irradiation.

5. This solution is transparent to ultraviolet light in the region 255–285 mμ. Irradiation with longer wavelengths increases the yield of lumisterol$_3$, while shorter wavelengths increase the yield of stachysterol$_3$. The chief by-product resulting from irradiation within this range is tachysterol$_3$, which is separated easily by forming an addition product with maleic anhydride.[6]

6. A reproducible and constant velocity (100–150 l. per hour) is established by maintaining a glass float at a given level in the vertical feed tube.

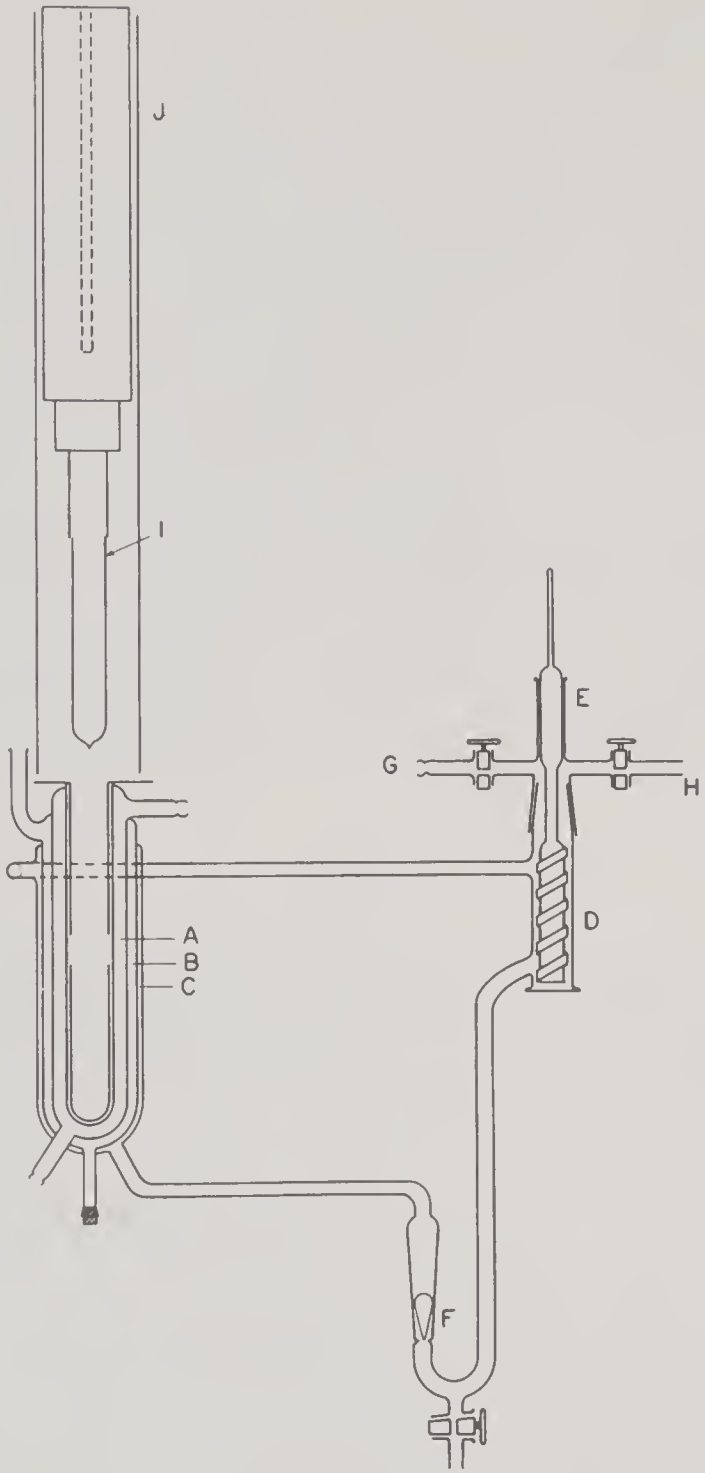


Fig. XIV, 1. Irradiation apparatus (E. Havinga and J. P. L. Bots). A, circulating water; B, sodium nitrate solution; C, circulating sample solution; D, glass impeller; E, Pyrex bearing (glycerol lubricated) made from a syringe; F, glass float (velocity indicator); G, vacuum outlet; H, nitrogen inlet; I, mercury lamp; J, iron case.

7. Irradiation is stopped when only 44.5% of the starting material is converted, in order to minimize the formation of over-irradiation products which are not readily separated from the product.

8. A colorimetric determination with Nield's reagent shows the crude product to contain 47% vitamin D_3 and 25% tachysterol$_3$, (d).

9. The saponified condensation product of tachysterol$_3$-3-C^{14}, (d), and maleic anhydride is alkali-soluble; see Havinga.[4]

10. A crystalline by-product does not dissolve.

11. Vitamin D$_3$ 3,5-dinitrobenzoate, crystallized from acetone-methanol, melts at 128–129°; a modification[1] (m.p. 133–134°) is obtained by recrystallization from ether.

12. The yield is based on colorimetric assay. Trial preparations have shown that the dry weight yield is about 20% greater, the chief by-product lumisterol$_3$-3-C^{14}, (e). Pure lumisterol$_3$ melts[4] at 88–89°. Pure vitamin D$_3$ crystallizes from dilute acetone, m.p. 84–85°, and is stored best in evacuated amber vials at 0°. The ultraviolet absorption spectra are given by Havinga and Bots.

Tracer studies[4] indicate that lumisterol plays no part as an essential intermediate in the conversion of 7-dehydrocholesterol [-3-C^{14}] to tachysterol$_3$,(d), and vitamin D$_3$ under the conditions employed.

[1]J. A. K. Buisman, W. Stevens and J. v. d. Vliet, Rec. trav. chim., *66*, 83 (1947).

[2]E. Lederer and M. Lederer, *Chromatography*, Elsevier, New York, 1953, p. 22.

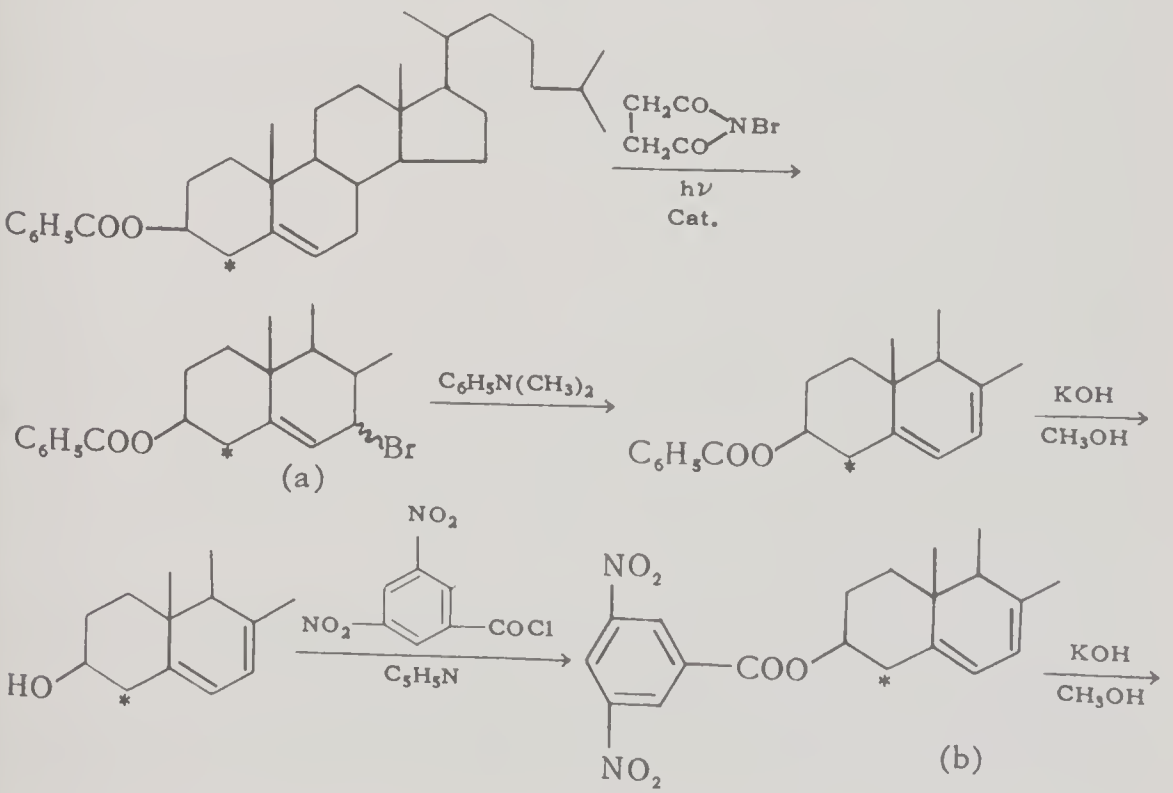
[3]D. F. Louw, J. Strating and H. J. Bocker, Rec. trav. chim., *74*, 1540 (1955).

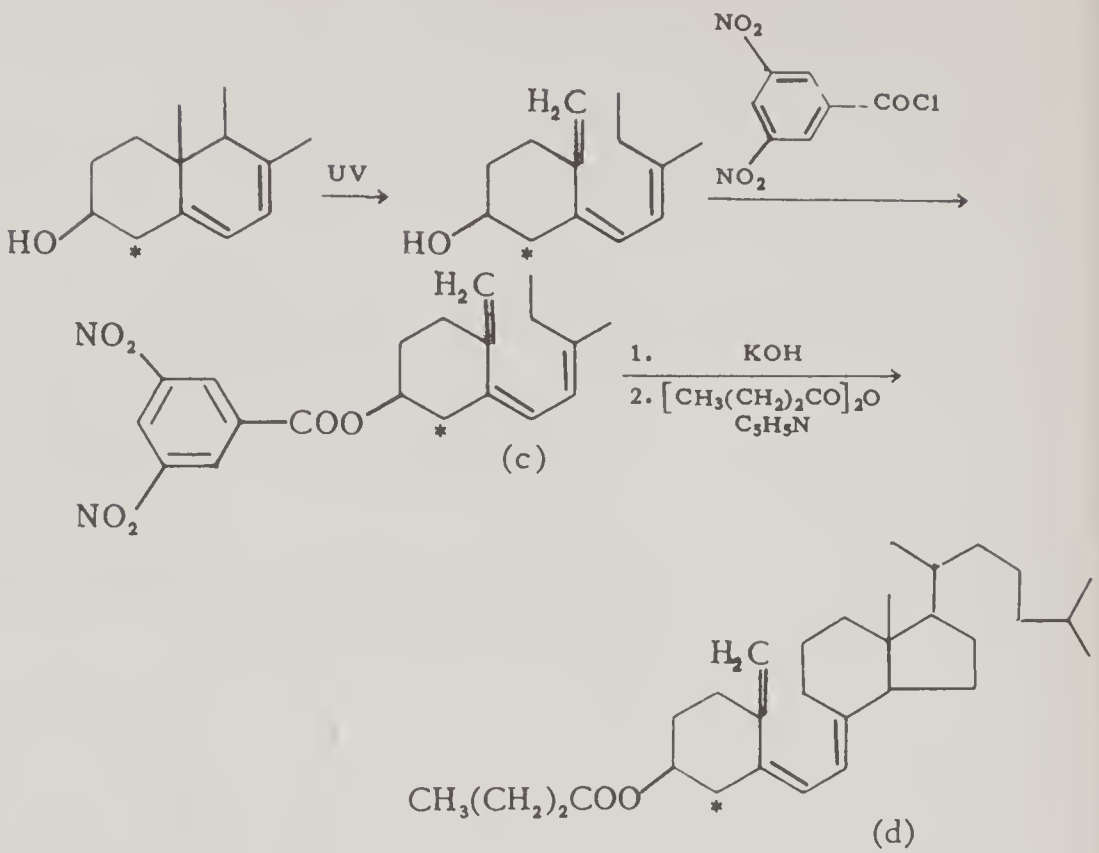
[4]E. Havinga, A. L. Koevoet and A. Verloop, Rec. trav. chim., *74*, 1230 (1955).

[5]E. Havinga, A. L. Koevoet and A. Verloop, Rec. trav. chim., *74*, 788 (1955); Chem. Weekblad, *50*, 803 (1954).

[6]*Organic Reactions*, Vol. IV, Wiley, New York, 1948, p. 48.

VITAMIN D$_3$-4-C^{14}





F. Hunziker, Helv. Chim. Acta, 38, 917 (1955).

A. Procedure (Note 1)

(a) *7-Bromocholesteryl-4-C*^{14} *Benzoate* (Note 2). To a solution of 3.92 g. (8 mmoles) of cholesteryl-4-C^{14} benzoate in 20 ml. of warm petroleum ether (b.p. 50–70°) and 20 ml. of ligroin (b.p. 70–110°), heated under reflux at 85° under anhydrous conditions, is added 1.70 g. (9.6 mmoles) of *N*-bromosuccinimide and 0.005 g. of benzoyl peroxide catalyst during 10 minutes. At 18 minutes (Note 3) the mixture is cooled to 20°, and unreacted cholesteryl-4-C^{14} benzoate is collected on a filter, confirming the indications of incomplete reaction. The unreacted starting material is dissolved in a large volume of petroleum ether, and the combined filtrates are evaporated under vacuum at 35°. A mixture of the residue, 0.85 g. of *N*-bromosuccinimide, some benzoyl peroxide, 20 ml. of petroleum ether and 20 ml. of ligroin is refluxed for 7 minutes under the illumination[1] of a 250-W incandescent lamp. The mixture is cooled to 10°, and the precipitate is collected and washed with cold petroleum ether. The filtrate is evaporated under vacuum at 35°, giving 5.04 g. of crude semicrystalline product.

(b) *5,7-Cholestadien-3β-yl-4-C*^{14} *3,5-Dinitrobenzoate*, (*7-Dehydrocholesteryl-4-C*^{14} *3,5-Dinitrobenzoate.*) The 7-bromocholesteryl-4-C^{14} benzoate is heated at 140° for 1 hour under vacuum with 25 ml. of *N,N*-dimethylaniline, freshly distilled over sodium. The mixture is distributed between ether and an excess of ice-cold, dilute hydrochloric acid, and

the extract is washed with three portions of hydrochloric acid-ice and water, dried and evaporated. The residue consists of 5,7-cholestadien-3β-yl-4-C^{14} benzoate and the dehydrobromination by-products: 4,6-cholestadien-3β-yl-4-C^{14} and 2,4,6-cholestatriene-4-C^{14}.

Saponification is accomplished by heating the residue for 45 minutes under nitrogen with 40 ml. of benzene and 40 ml. of 5% methanolic potassium hydroxide. The residue, obtained by evaporation, is distributed between water and freshly distilled ether, and the extract is washed with four portions of water, dried over sodium sulfate and evaporated, giving 3.45 g. of crude 5,7-cholestadien-3β-ol-4-C^{14}.

To a stirred solution of the crude saponification product in 25 ml. of dry pyridine is added 3.8 g. of powdered 3,5-dinitrobenzoyl chloride. The mixture is stored in the dark at room temperature for 63 hours under vacuum, then diluted with 50 ml. of methanol and stored for 2 hours. The product is collected, washed with three portions each of methanol and cold acetone, and recrystallized from ethyl acetate; yield 1.82 g. (41%), m.p. 205–210° (dec.). A second crop (0.10 g.) is obtained from the mother liquor.

(c) *Vitamin D_3-4-C^{14} 3,5-Dinitrobenzoate.* A mixture of 2.25 g. of 5,7-cholestadien-3β-yl-4-C^{14} 3,5-dinitrobenzoate, 12 ml. of dioxane (freshly distilled over sodium) and 9 ml. of freshly prepared 5% methanolic potassium hydroxide is shaken for 1.5 hours at room temperature under reduced pressure. The partially crystalline material is distributed between ether and water, and the extract is washed with water, dried over sodium sulfate and evaporated under vacuum; yield 1.51 g. of 5,7-cholestadien-3β-ol-4-C^{14}.

The diene is stored under vacuum for 3 hours in an irradiation apparatus (Note 4). The apparatus is flushed twice with nitrogen, and the compound is dissolved by the addition of 2 l. of freshly distilled peroxide-free ether. The solution is irradiated for 60 minutes and evaporated. A cooled solution of the residual crude vitamin D_3-4-C^{14} (1.57 g. of yellow resin) in 5 ml. of dioxane (freshly distilled over sodium) and 5 ml. of pyridine is treated with 1.5 g. of powdered 3,5-dinitrobenzoyl chloride. The mixture is stored under vacuum in the dark for 63 hours and diluted to 100 ml. with ice-water. The yellow precipitate is caused to crystallize by stirring for a few hours, then is collected and washed with water and with cold ether, which is filtered into potassium bicarbonate solution and ice (Note 5). The ether extract is washed with three portions of potassium bicarbonate solution, dried over sodium sulfate and evaporated. The residue (1.95 g.) is dissolved in petroleum ether, and the filtered solution (Note 6) is separated by chromatography on a column of 60 g. of aluminum oxide (Note 7) and elution with 2500 ml. of petroleum ether (Note 8). Evaporation gives 1.40 g. of yellow resin which then is crystallized from 10 ml. of petroleum ether at –10°. The crude product

is collected, washed with cold petroleum ether and separated from some further 5,7-cholestadien-3β-yl-4-C^{14} 3,5-dinitrobenzoate by treatment with a little cold ether. The filtrate is evaporated, and the residue is recrystallized from petroleum ether; yield 0.40 g. (20%), m.p. 131.5–132.5°, $[\alpha]_D^{25}$ + 93.3 ±2° (*c*, 0.564 $CHCl_3$). Additional crops are obtained from the mother liquor: 0.04 g., m.p. 131–133°, $[\alpha]_D^{23.5}$ + 89.5 ±2° (*c*, 0.520 $CHCl_3$); 0.06 g. (not further characterized). The molar specific activity agrees with the expected value, and the rotation corresponds to a purity of about 90%.

(d) *Vitamin D_3-4-C^{14} Butyrate* (Note 9). A mixture of 0.115 g. of vitamin D_3-4-C^{14} 3,5-dinitrobenzoate, 4 ml. of dioxane (freshly distilled from sodium) and 4 ml. of freshly prepared 5% methanolic potassium hydroxide is stored under vacuum for 1 hour at room temperature. The reddish-violet solution is diluted with freshly distilled ether and water, and the ether extract is dried over sodium sulfate and evaporated.

The vitamin D_3-4-C^{14} (0.085 g.) is treated with 3 ml. of dry pyridine and 1 ml. of distilled butyric anhydride. The mixture is stored under vacuum for 63 hours, then diluted with a little methanol, stored for another hour under vacuum at room temperature and finally heated on a water-bath. The residue is distributed between ice-water and ether, and the ether solution is washed three times with dilute hydrochloric acid-ice, twice with water, three times with potassium bicarbonate-ice, once with brine and then dried over sodium sulfate and evaporated. The residue (0.20 g.) is dissolved in petroleum ether (Note 6) and passed through a column of 6.0 g. of aluminum oxide (Note 10) by washing with 120 ml. of petroleum ether. Evaporation gives 0.159 g. of colorless oil, which is crystallized from cold concentrated acetone solution by the cautious addition of cold methanol and cooling to –10°. The product is dissolved in petroleum ether, the solution is evaporated, and the residue is again crystallized from acetone-methanol; yield 0.043 g. (45%), m.p. 62–64°; $[\alpha]_D^{25}$ + 39.2 ±2°(*c*, 0.35 $CHCl_3$), $E_{1cm.}^{1\%}$: 343.1, 390.2 and 329.4 respectively, for 255, 265 and 273 mμ (cyclohexane), purity 92% (Note 11).

B. Notes

1. The procedure is similar to that of Havinga and Bots; see Vitamin D_3-3-C^{14}.

2. The method is that of Ziegler.[2]

3. In trial experiments the bromination was complete at 10 minutes without illumination. Hunziker suggests that the free radical mechanism[3] in the nonpolar solvent might have been hindered by the β-particle emitted by C^{14} (less than 1 μc.).

4. An apparatus is described by Havinga and Bots (Note 1).

5. The insoluble residue is washed with methanol and acetone, giving

0.38 g. of quite pure 5,7-cholestadien-3β-yl-4-C^{14} 3,5-dinitrobenzoate.

6. A small amount of insoluble material is removed.

7. Aluminum oxide (Merck) is activated by heating at 150° for 45 minutes under vacuum. An aliquot (10 g.) stirred with 100 ml. of water for 15 minutes has pH 4.3.

8. The by-products, lumisterol-4-C^{14} 3,5-dinitrobenzoate and 5,7-cholestadien-3β-ol-4-C^{14}, are slower moving on the column.

9. The stability of the butyric ester is greater than that of the free vitamin D_3.

10. Aluminum oxide (Merck) is "inactivated" by stirring with successive portions of boiling methanol and ethyl acetate, filtering, and heating under vacuum for 3 hours on a steam-bath. An aliquot (10 g.) stirred with 100 ml. of water for 15 minutes has pH 6.0.

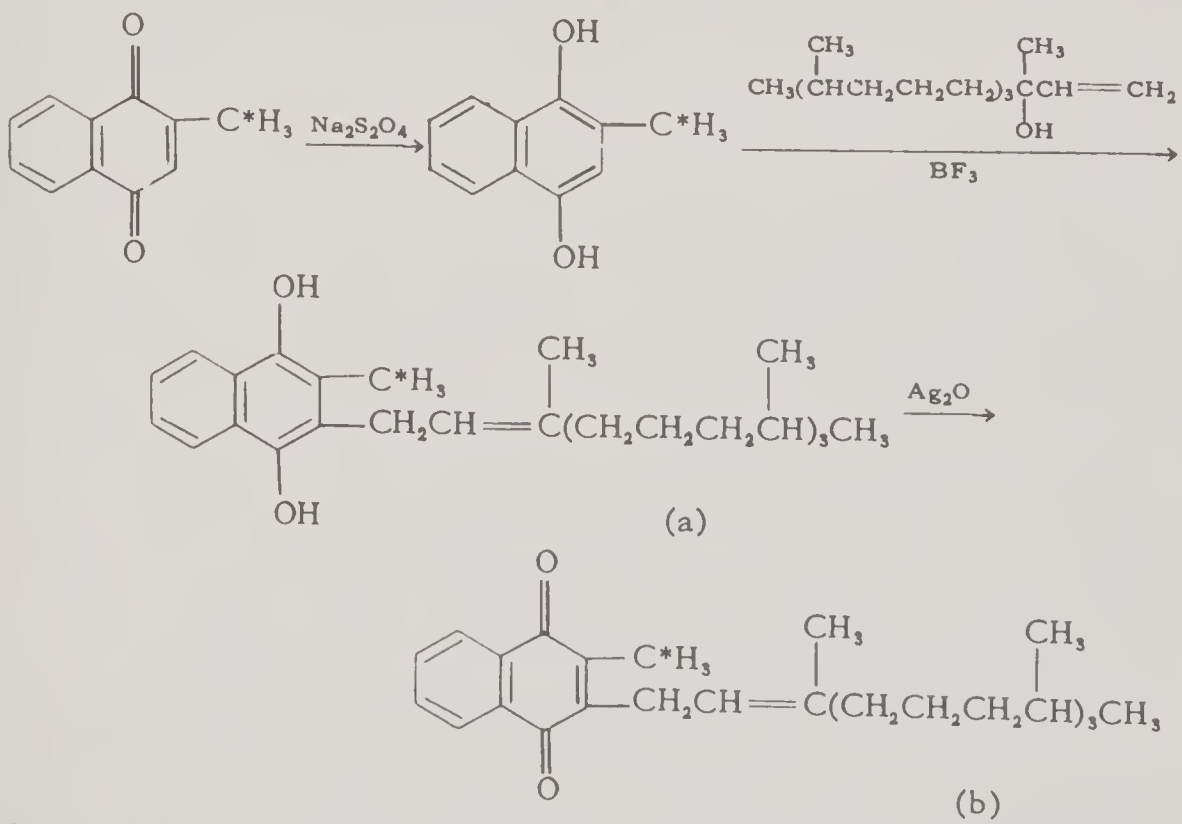
11. Pure vitamin-D_3 butyrate, m.p. 63–65°, $[\alpha]_D^{25}$ + 39° ($CHCl_3$), λ max. 265 mμ, $E_{1cm.}^{1\%}$ 423.

[1]Ch. Meystre, L. Ehmann, R. Nehr and K. Miescher, Helv. Chim. Acta, *28*, 1252 (1945).

[2]K. Ziegler, A. Späth, E. Schaaf, W. Schumann and E. Winkelmann, Ann., *551*, 80 (1942); A. E. Bide, H. B. Henbest, E. R. H. Jones, R. W. Peevers and P. A. Wilkinson, J. Chem. Soc., *1948*, 1783.

[3]C. F. Bloomfield, J. Chem. Soc., *1944*, 114.

2-METHYL-C^{14}-3-PHYTYL-1,4-NAPHTHOQUINONE (C_1^{14}-Vitamin K_1)



C. C. Lee, F. C. G. Hoskin, L. W. Trevoy, L. B. Jaques and J. W. T. Spinks, Can. J. Chem., *31*, 769 (1953).

A. Procedure (Note 1)

(a) *2-Methyl-C^{14}-3-phytyl-1,4-naphthalenediol.* A mixture of 0.500 g. of 2-methyl-C^{14}-1,4-naphthalenediol (Note 2), 0.400 g. of isophytol, 0.1 ml. of 45% boron trifluoride and 2 ml. of anhydrous dioxane is shaken in a small stoppered flask under nitrogen for 24 hours at room temperature. The mixture is diluted with 20 ml. of water and washed with 10 ml. of water and three 10-ml. portions of 2% potassium hydroxide solution containing 5% sodium hydrosulfite. The combined alkaline solutions are extracted with ether, and the extract is washed with alkali (Note 3), dried over sodium sulfate and evaporated under reduced pressure. The residue is hydrogenated in 3–4 ml. of petroleum ether over 0.1 g. of a palladium-on-calcium carbonate[1] catalyst (Note 4) for 30 minutes at room temperature and atmospheric pressure. The hydroquinone of vitamin K_1 is transferred under nitrogen into a 15-ml. centrifuge cone, with a few ml. of cold petroleum ether, and crystallized by cooling in an ice-salt bath. The solid is separated by centrifuging and washed with several small portions of cold petroleum ether. The product is dissolved in 10 ml. of ether, and the catalyst is removed.

(b) *2-Methyl-C^{14}-3-phytyl-1,4-naphthoquinone.* The ethereal solution of the hydroquinone is stirred for 30 minutes with 0.50 g. of silver oxide. The mixture is dried over sodium sulfate, and the solution is separated by centrifugation and evaporated to give 0.146 g. of product (Note 5). The yield is 20% based on the reacted 2-methyl-C^{14}-1,4-naphthoquinone; the molar specific activity is the same as that of the starting material.

B. Notes

1. The preparation is adapted from the method of Isler.[2]

2. The starting material is prepared by reducing 2-methyl-C^{14}-1,4-naphthoquinone with sodium hydrosulfite by the procedure of Fieser.[3]

3. The unreacted 2-methyl-C^{14}-1,4-naphthalenediol (0.224 g.) is recovered by ether extraction of the acidified washings. The extract is washed with 10% sodium hydrosulfite solution, dried over sodium sulfate, concentrated to about 1 ml., cooled and filtered to remove precipitated sulfur, which is washed with a small amount of cold ether. The combined solutions are diluted with petroleum ether to precipitate a gray powder, which is collected and purified by dissolution in ether and repetition of the entire process. This material is suitable for vitamin K_1 synthesis. Oxidation with silver oxide in ether forms 2-methyl-1,4-naphthoquinone, which is isolated by sublimation, m.p. 103–104°.

4. The catalyst will not reduce double bonds. This procedure is used in the commercial process to eliminate oxidative loss during isolation of the hydroquinone.

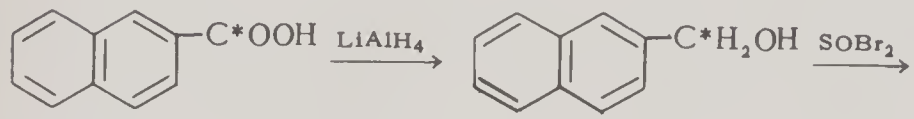
5. The product is a clear, yellow oil, exhibiting an ultraviolet absorption spectrum that is practically identical with that of an authentic sam-

ple. Reductive acetylation[4] with zinc, acetic anhydride and a small amount of pyridine forms the colorless diacetate, m.p. 59–60°

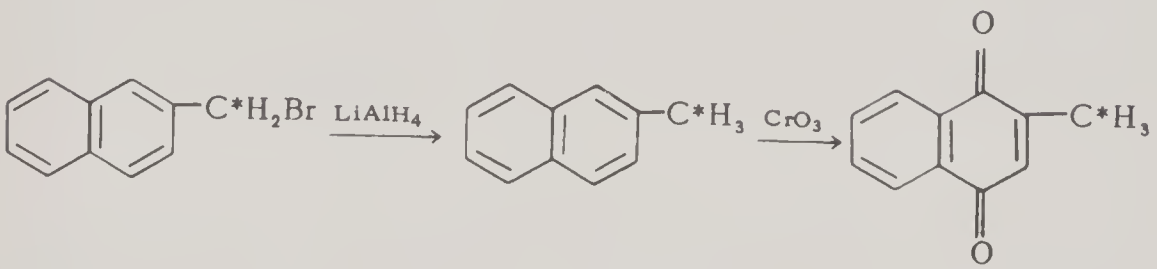
[1]H. Lindlar, Helv. Chim. Acta, *35*, 446 (1952).
[2]O. Isler and K. Doebel, Belg. 512,573.
[3]L. F. Fieser, J. Biol. Chem., *133*, 391 (1940).
[4]L. F. Fieser, J. Am. Chem. Soc., *61*, 3467 (1939).

2-METHYL-C^{14}-1,4-NAPHTHOQUINONE (C_1^{14}-Vitamin K_3)

METHOD I



(a)



(b) (c)

R. V. Phillips, L. W. Trevoy, L. B. Jaques and J. W. T. Spinks, Can. J. Chem., *30*, 844 (1952).

A. Procedure

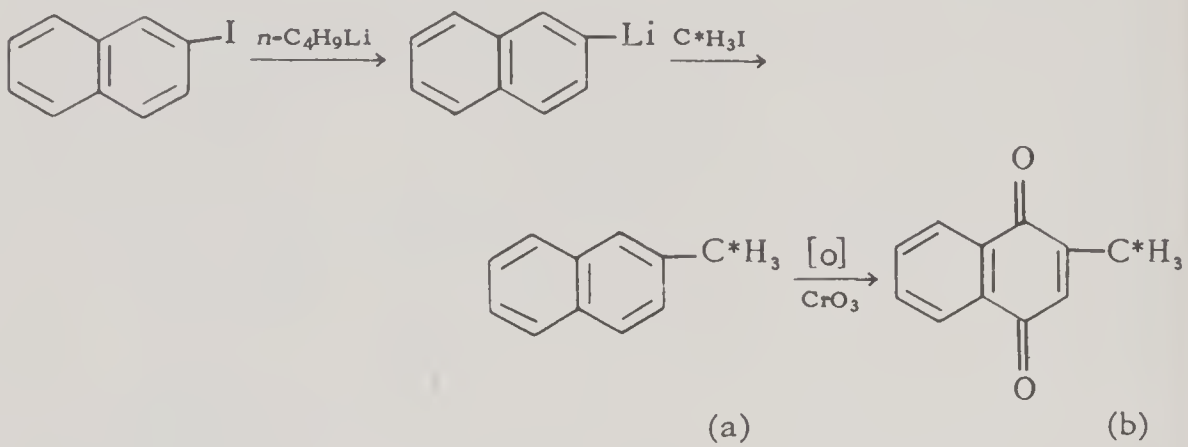
(a) *2-Naphthalenemethanol-α-C^{14}.* 2-Naphthoic-C^{14} acid (0.230 g.) is reduced in ether solution by addition to 1.4 mmoles of lithium aluminum hydride[1] in ether. The colorless product is recrystallized from benzene-petroleum ether; yield 0.183 g. (97%), m.p. 79–79.5°.

(b) *2-Methyl-C^{14}-naphthalene.* To a solution of 0.182 g. of 2-naphthalenemethanol-α-C^{14} in 5 ml. of ether at −80° is added 0.27 g. of thionyl bromide, and the mixture is set aside for 1.5 hours at room temperature. Reduction of the 2-(bromomethyl-C^{14})naphthalene is effected by slowly adding the mixture with cooling to 4.0 mmoles of lithium aluminum hydride in ether, according to the method of Trevoy and Brown.[2] The product is recovered from the ether solution and sublimed at 50–80° (20 mm.); yield 0.123 g. (71%), m.p. 33.5–34°.

(c) *2-Methyl-C^{14}-1,4-naphthoquinone, (Menadione-α-C^{14}).* To a solution of 0.107 g. of 2-methyl-C^{14}-naphthalene in 1 ml. of glacial acetic acid is added 0.75 ml. of a chromic oxide solution, prepared by dissolving 7 g. of chromic anhydride in a mixture of 7 ml. of water, 4.2 ml. of glacial

acetic acid and 0.66 ml. of concentrated sulfuric acid. The reaction mixture is shaken at 50° for 5 minutes, then diluted with 4 ml. of cold water and cooled. The product is collected, washed with several small portions of cold water and recrystallized from methanol; yield 0.0516 g. (40–42%), m.p. 103.5–104.5° (see Method II, and 2-methyl-1,4-naphthoquinone-4-C^{14}).

METHOD II



(a) (b)

A. Murray III and A. R. Ronzio, J. Am. Chem. Soc., 74, 2408 (1952).

A. Procedure

(a) *2-Methyl-C^{14}-naphthalene* (Note 1). To a stirred solution of 3.08 g. (12.13 mmoles, 50% excess) of 2-iodonaphthalene in 50 ml. of absolute ether, maintained at −30° (Note 2) under dry nitrogen, is added an ethereal solution of 8.08 mmoles of butyllithium during 2.5 minutes. The mixture is stirred for 30 minutes (Note 3) and frozen with liquid nitrogen; then the system is evacuated to 10 μ, and 0.5735 g. (4.04 mmoles, 50% of the calculated amount) of methyl-C^{14} iodide is added by distillation. The vacuum is replaced by slightly less than one atmosphere of nitrogen, the flask is closed, and the mixture is allowed to warm to no more than 0° and is stirred for 3 hours. To the stirred mixture, again cooled to −30°, is added 6.05 mmoles (35–50% excess) of butyllithium during 2 minutes, and stirring is continued for 30 minutes. The mixture is cooled to −60°, carbonated for 20 minutes with dry gaseous carbon dioxide and hydrolyzed with 50 ml. of water. The product is steam-distilled (Note 4) from an alkaline ether-water mixture into a 1-l. liquid-liquid extractor, and isolated by continuous extraction for 28 hours with a minimal volume of ether (Note 5). The extract (20 ml.) is refluxed for 16 hours with 15 ml. of distilled piperidine in a system closed with a guard tube filled with potassium hydroxide pellets (Note 6). The solution is acidified cautiously with 3 *N* hydrochloric acid and extracted with ether for 5.5 hours. The extract is dried over Drierite, treated with carbon, filtered and concentrated by distillation through a column (Note 7).

(b) *2-Methyl-C^{14}-1,4-naphthoquinone,* (*Menadione-α-C^{14}*), (Note 8). To a stirred solution of the 2-methyl-C^{14}-naphthalene, 21 ml. of glacial acetic acid, 0.38 ml. of concentrated sulfuric acid and 1.3 ml. of water, maintained at 0° in the dark, is added a solution of 7.00 g. of chromic oxide, 10 ml. of water, 9 ml. of glacial acetic acid and 0.38 ml. of sulfuric acid over a period of 9.5 minutes. At 18 minutes, the ice-bath is replaced by a water-bath at 17°, and the mixture is stirred for 3 hours, then poured slowly onto a mixture of 200 g. of crushed ice, 200 ml. of water and 200 ml. of ether in a 4-l. beaker. The pH is adjusted to a point just acidic to litmus, by the cautious addition of 42 g. of sodium bicarbonate in 450 ml. of water, and the mixture is continuously extracted with ether for 11 hours, then for 12.5 hours with fresh ether (Note 9). The extract is evaporated in a vacuum sublimator, and the crude product (0.5334 g., 76.7% based on methyl iodide) is sublimed at 57° (10 μ) for 3.5 hours (Note 10). Purification is accomplished by successive recrystallization at −80° from petroleum ether (35 ml.), hexane (13.5 ml.), petroleum ether (24 ml.), methanol (9.5 ml.), and methanol (6 ml., Note 11), followed by resublimation, treatment with carbon and recrystallization from 12 ml. of methanol at −30°. The yield is 0.3380 g. (48.7%), m.p. 106–106.5°.

B. Notes

1. The method is a modification of the methods of Gilman[3] and Vesely.[4] The preparation of 2-naphthyllithium by the halogen-metal replacement reaction (49–55% yield) leads to the formation of considerable naphthalene, not easily separated from 2-methylnaphthalene; the same hydrocarbon is obtained by the Grignard reaction. The only by-products of the preparation by the halogen-metal interconversion reaction are 2,2′-binaphthyl, butyl iodide and a trace of 2-naphthyl ethyl ether, all easily removed.

2. The formation of 2,2′-binaphthyl begins at 5–10° and is greatly diminished in the cold. At-60°, however, the interconversion reaction is too slow.

3. The yield of 2-naphthyllithium at −25°, determined by carbonation, is constant for a reaction time of 10–60 minutes, 85.2–87.9%. The solutions are stable for 24 hours at 0° and no naphthalene is formed.

4. From the residue has been isolated fluorescent (U.V.) 2,2′-binaphthyl (m.p. 180–181°) and 2-naphthyl ketone (m.p. 165–166°).

5. The aqueous solution possesses very little activity.

6. As the butyl halides interfere in the subsequent oxidation step, they are removed by salt formation with piperidine, by a modification of the method of Semb.[5]

7. The yield of 2-methylnaphthalene is 95–100% based on methyl iodide.

8. The procedure is a modification of the method described by Lucas and Pressman.[6] Oxidation in a medium of carbon tetrachloride, by the method of Hyman and Peters,[7] gave incomplete reaction. As the quinone is unstable in light, all operations are carried out in subdued light in low-actinic glassware, or apparatus that is shielded with opaque tape. Solutions in ordinary glassware have been reported[8] to be 80% polymerized in 5 hours.

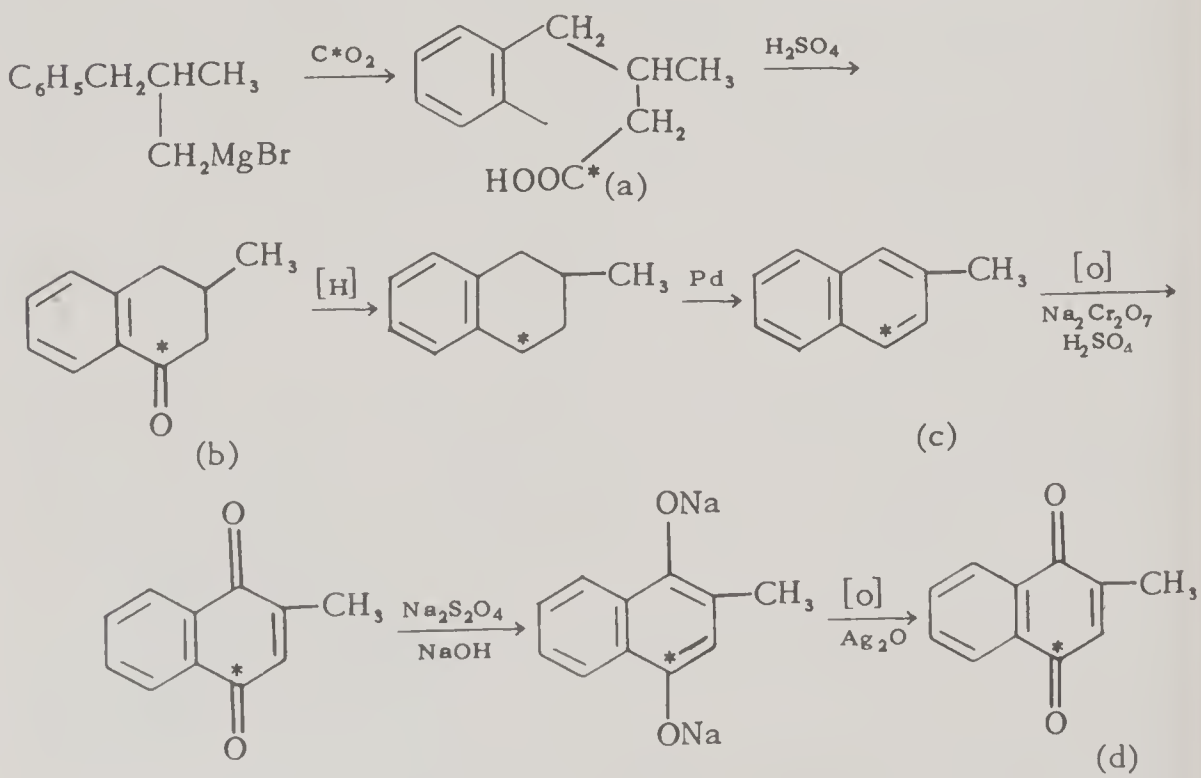
9. The recovery was 97.5% when pure 2-methyl-1,4-naphthoquinone was carried through the oxidation.

10. At 2.5 hours the sulbimation residue is redistributed by dissolution in ether.

11. M.p. 101–103°, 0.3565 g. (51.3% yield).

[1]R. F. Nystrom and W. G. Brown, J. Am. Chem. Soc., *69*, 2548 (1947).
[2]L. W. Trevoy and W. G. Brown, J. Am. Chem. Soc., *71*, 1677 (1949).
[3]H. Gilman and F. W. Moore, J. Am. Chem. Soc., *62*, 1843 (1940).
[4]V. Vesely and F. Storsa, Chem. Listy, *26*, 495 (1932); Chem. Abstracts, *27*, 717 (1933).
[5]J. Semb and S. M. McElvain, J. Am. Chem. Soc., *53*, 690 (1931).
[6]H. J. Lucas and D. Pressman, *Principles and Practice in Organic Chemistry*, Wiley, New York, 1949, p. 442.
[7]J. Hyman and C. F. Peters, U. S. 2,402,226; Chem. Abstracts, *40*, 6101 (1946).
[8]W. R. Collins and E. R. Kirch, J. Am. Pharm. Assoc., *35*, 215 (1946).

2-METHYL-1,4-NAPHTHOQUINONE-4-C[14]



L. Li and W. H. Elliott, J. Am. Chem. Soc., *74*, 4089 (1952).

A. Procedure

(a) *3-Methyl-4-phenylbutyric-1-C*^{14} *Acid.* To a Grignard reagent prepared (Note 1) from 1.5 g. (7.5 mmoles) of (3-bromo-2-methylpropyl)-benzene and 0.182 g. of acid-washed magnesium turnings is added the dry carbon-C^{14} dioxide from 0.987 g. of barium carbonate-C^{14} by distillation with liquid nitrogen. The cooling bath is replaced with another at −15°, and the mixture is stirred (magnetically) for 30 minutes, before being brought to atmospheric pressure with nitrogen. After 10 hours, the reaction mixture is hydrolyzed with dilute hydrochloric acid, and the ether layer is removed and extracted with 10% sodium hydroxide solution. The alkaline extract is acidified and extracted with ether. The extract is dried over magnesium sulfate, and the solvent is evaporated. The yield of colorless oil is 0.818 g., 91.5% (Note 2).

(b) *3,4-Dihydro-3-methyl-1(2H)-naphthalenone-1-C*^{14}, (*3-Methyl-1-tetralone-1-C*^{14}). To the acid at 0° is added with stirring 5.5 ml. of sulfuric acid (d. 1.84) over a period of 40 minutes. After standing at room temperature for 24 hours, the mixture is poured onto crushed ice, and the oily product is extracted with ether and washed with water, sodium bicarbonate and water. Removal of solvent leaves 0.625 g. (85%) of a slightly yellow oil (Note 3).

(c) *2-Methylnaphthalene-4-C*^{14}. 3-Methyl-1-tetralone-1-C^{14} (0.588 g.) in 6 ml. of toluene is reduced by the Clemmensen method for a period of 26 hours, using zinc amalgam, 6 ml. of 38% hydrochloric acid and 0.3 ml. of glacial acetic acid. The resulting yellow oil (0.455 g.) is heated at 250° for 3 hours over a palladium-charcoal catalyst (Note 4) in an 18 × 160 mm. Pyrex tube fitted with an air condenser. The product is extracted from the reaction mixture with ether, and solvent removal leaves 0.420 g. of solid, which is purified by sublimation; yield 0.330 g. (64%), m.p. 34.5° (Note 5).

(d) *2-Methyl-1,4-naphthoquinone-4-C*^{14}, (*Menadione-4-C*^{14}). A solution of 0.330 g. of 2-methylnaphthalene-4-C^{14} in 5.5 g. of carbon tetrachloride is added to a solution of 1.65 g. of sodium dichromate dihydrate in 0.8 ml. of water, contained in a 50-ml. flask fitted with a reflux condenser. The reaction mixture is stirred magnetically) at 50–55° while 1.7 ml. of 80% sulfuric acid is added dropwise. The temperature of the bath is raised gradually to 80° over a period of 10 minutes and held there for 5 minutes; then the flask is chilled with ice-water, and the aqueous layer is separated and extracted with carbon tetrachloride and ether. The combined extracts are extracted with several 5-ml. portions of a freshly prepared 2% solution of sodium hydrosulfite in sodium hydroxide (2%) until no color remains (Note 6). The hydrocarbon remaining in the carbon tetrachloride is reoxidized (as above) with 1 g. of sodium dichromate in 0.5 ml. of water and 1.0 ml. of 80% sulfuric acid. The 2-methyl-1,4-naphthalenediol-4-C^{14} (0.225 g.) from both oxidations is oxidized with

0.450 g. of silver oxide in the presence of a small amount of anhydrous magnesium sulfate. The crude product, 0.212 g., m.p. 98–100°, is vacuum-sublimed to give 0.185 g. of product melting at 104.5–105° (Note 7); yield 50% (Note 8).

B. Notes

1. The Grignard reagent is prepared in an atmosphere of nitrogen, as usual, in the typical apparatus described by Calvin.

2. A trial preparation was distilled at 127° (0.1 mm.).

3. The oxime melts at 123°. See 7-methyl-1-tetralone-1-C^{14} for a similar ring closure.

4. The preparation of catalyst for vapor phase dehydrogenation is described by Linstead.[2]

5. The m.p. of the picrate (116°) is not depressed upon mixed melting with an authentic sample.

6. The yellow quinone is reduced to a water soluble salt of hydroquinone.

7. There is no depression of the melting point upon mixed melting with an authentic sample of 2-methyl-1,4-naphthoquinone. Recrystallization from 50% methanol raises the melting point to 106.5°.

8. Oxidation to the quinone by a modification of the method of Hyman and Peters[3] is believed to be more satisfactory than by a modification of the method of Fieser,[4] the yields being 48–52% and 37–41%, respectively. However, see 2-methyl-C^{14}-1,4-naphthoquinone, Method II.

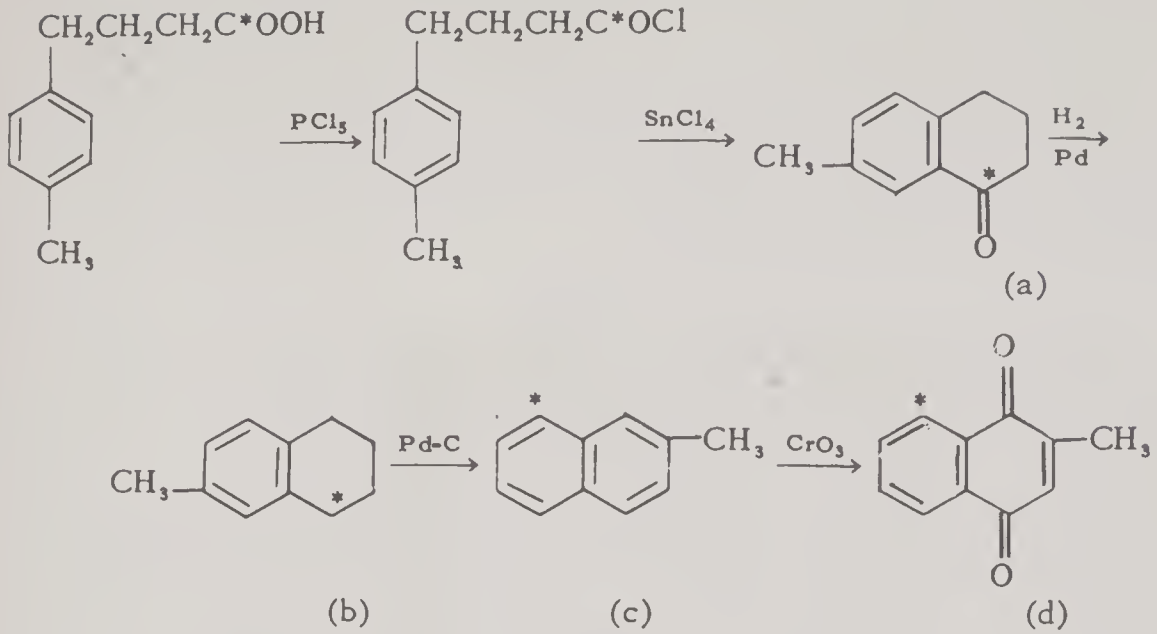
[1]M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon*, Wiley, New York, 1949, p. 142.

[2]R. P. Linstead and S. L. S. Thomas, J. Chem. Soc., *1940*, 1127.

[3]J. Hyman and C. F. Peters, U. S. 2,402,226; through Chem. Abstracts, *40*, 6101 (1946).

[4]L. F. Fieser, J. Biol. Chem., *133*, 391 (1940).

2-METHYL-1,4-NAPHTHOQUINONE-8-C^{14}



C. J. Collins, J. Am. Chem. Soc., 73, 1038 (1951).

A. Procedure

(a) *3,4-Dihydro-7-methyl-1(2H)-naphthalenone-1-C^{14}*, (*7-Methyl-1-tetralone-1-C^{14}*). Ring closure of the acid chloride prepared from 1.26 g. of 4-*p*-tolylbutyric-1-C^{14} acid is effected by a suitable adaptation of the stannic chloride method of Bachmann and Thomas,[1] which follows (for the preparation of the methoxyl compound). The yield is 91% (Note 1).

To a cold solution of 21.5 g. of 4-(*m*-methoxyphenyl)butyric acid in 100 ml. of benzene is added 27.7 g. of finely powdered phosphorus pentachloride, and the mixture is set aside at room temperature for 1–2 hours. The acid chloride, after cooling in an ice-bath, is cyclized by treatment with a cold solution of 19 ml. of anhydrous stannic chloride in 50 ml. of benzene. After standing for 10 minutes in the ice-bath, the slurry is poured onto ice; then concentrated hydrochloric acid is added to effect solution, and the product is extracted into ether. The extract is washed with dilute acid, water, dilute sodium hydroxide and water, then dried, concentrated and vacuum-distilled. The 7-methoxy-1-tetralone (18.7 g., 96% yield, b.p. 130–135° at 0.7 mm.) is recrystallized from petroleum ether-acetone.

(b) *7-Methyl-1,2,3,4-tetrahydronaphthalene-1-C^{14}*. The 3,4-dihydro-7-methyl-1(2H)-naphthalenone-1-C^{14} is reduced (91% yield) by an adaptation of the method of Kindler.[2] A molecular compound with sulfuric acid in acetic acid is hydrogenated over palladium at 3.5 atmospheres at room temperature.

(c) *2-Methylnaphthalene-8-C*^{14}. The 7-methyl-1,2,3,4-tetrahydronaphthalene-1-C^{14} is dehydrogenated over 30% palladium-on-carbon, yielding 0.57 g. of product (Note 2).

(d) *2-Methyl-1,4-naphthoquinone-8-C*^{14}. 2-Methylnaphthalene-8-C^{14} is oxidized with chromic oxide; see 5,6-chrysenedione-5,6,11,12-$C^{14}_{1/4}$ and 2-methyl-1,4-naphthoquinone-4-C^{14}. The yield of material melting at 104–105° is 52–55% (Note 3).

B. Notes

1. Pure 3,4-dihydro-7-methyl-1(2*H*)-naphthalenone melts[3] at 32.5–33.5°, n_D^{35} 1.55674, n_D^{20} 1.563; the semicarbazone melts at 224–225°. The yield by cyclization of the acid with sulfuric acid[2] is 66%.

2. The preparation of the catalyst and its use in the dehydrogenation of methyltetralins in the liquid phase is described by Linstead.[4] The evolution of hydrogen is 97% in 320 minutes, when 6-methyltetralin is boiled with 10% palladised charcoal; yield 78%,[4] m.p. 32.5°.

3. Reductive acetylation yields 2-methyl-1,4-naphthalenediol-8-C^{14} diacetate, m.p. 109.5–111.5°, having the same molar specific activity.

Oxidation[5] of the quinone with hot alkaline permanganate, followed by acidification, ether extraction, evaporation and vacuum sublimation, gives phthalic-3-C^{14} acid in 40–50% yield. Decarboxylation,[6] by heating the acid for 12 hours at 325°, gives a quantitative yield of benzoic-2,3-$C^{14}_{1/2}$ acid.

[1]W. E. Bachmann and D. G. Thomas, J. Am. Chem. Soc., *64*, 94 (1942).
[2]K. Kindler and D. Kwok, Ann., *554*, 9 (1944).
[3]F. Krollpfeiffer and W. Schafer, Ber., *56*, 620 (1920).
[4]R. P. Linstead and S. L. S. Thomas, J. Chem. Soc., *1940*, 1127; R. P. Linstead, A. F. Millidge, S. L. S. Thomas and A. L. Walpole, *ibid.*, *1937*, 1146.
[5]C. J. Collins, Nucleonics, 7 (4), 48 (1950).
[6]C. J. Collins, *ibid.*, 7 (4), 44 (1950).

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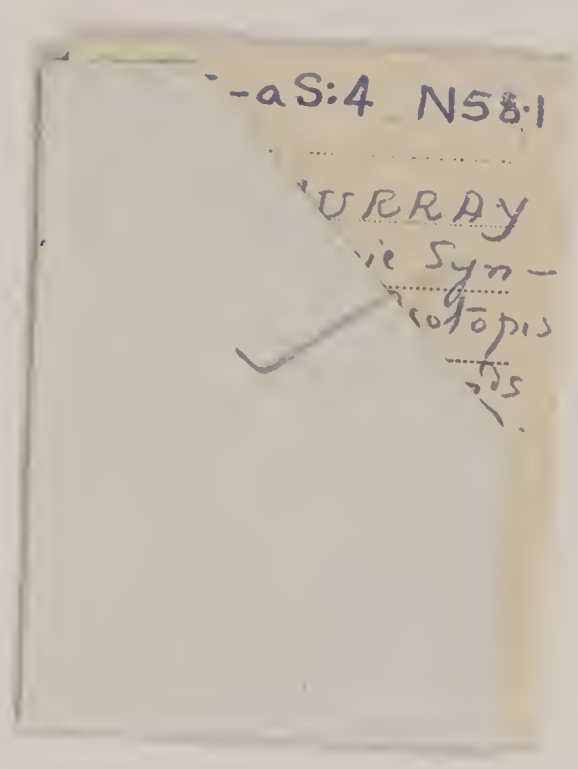
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